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THE TRYPETHELIACEAE OF MISSISSIPPI [1]

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(WITH 6 FIGURES)

Stromatic pyrenocarp lichens whose perithecia open by an apical pore have been assigned to the family Trypetheliaceae (Smith, **1921**; Zahlbruckner, **1926**). These lichens occur primarily on the bark of living trees throughout the tropical and subtropical regions of the world. Only three species have previously been cited from Mississippi, and one of these must be regarded as doubtful to the state (see below). Three additional species have been added by the present work (Johnson, **1955**). Hence our current knowledge of the Mississippi flora includes five valid and one doubtful species of the Trypetheliaceae. Since no extensive collections of this family exist from the United States, this study fills one gap from the relatively large area where the group occurs. All county records cited provide new data on distribution within the state. Most field notes included (e.g., the range of substrates on which each species occurs, etc.) also represent new information since few students of the group have been able to observe many specimens or many species in the field.

This study is based primarily on collections obtained by the writer during seven separate collecting trips to Mississippi extending over a period of fifteen years. Limiting this initial study to a single state has made possible more adequate field coverage than would otherwise have been the case. Mississippi is typical of the middle Gulf Coast areas of

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the United States and species found here should occur at similar latitudes in neighboring states. Some interest also develops from the distribution pattern of Trypetheliaceae within this area, perhaps correlated with the slight climatic transition from the subtropical regions on and near the coast to more temperate regions in the north. Members of the family are fairly abundant in southern Mississippi; they become less common to the north, and three of the five valid species have not yet been collected in northern counties.

The thallus of the Trypetheliaceae is embedded in the substrate (bark of living trees). Its area is noted primarily because the algal cells usually impart a somewhat different color or texture to the region of the bark where they occur. The thallus is divided into three layers: (1) the cortex (the outer layer), varying from a rather amorphous crust to strands of hyphae intertwined among bark cells; (2) the gonidial (middle) layer, composed principally of algal cells (*Trentepohlia*), which are readily stained and easily seen; and (3) the medulla (the inner layer), where bark cells are usually much more prominent than fungus cells.

Development of the perithecia and stroma is best known for *Melanotheca aggregata* (Johnson, **1940**). In this species perithecial initials arise as separate units and grow to about 12 μ in diameter before visible differentiation takes place. Blackening of the bark above the perithecial primordium usually accompanies the earliest stages. Several ascogonia develop within each perithecial cavity; the ascogonia form definite coils and are provided with trichogynes. Spermagonia are present, but there is no positive proof that they are functional. Ascogenous hyphae are formed and the perithecial cavity develops with the lichen digesting the necessary space among the bark cells. As the cavity increases in size, the bark cells surrounding the cavity and those extending both upward to the surface and laterally for a certain distance lose their normal appearance, becoming shrunken, dark, and carbonaceous. Since several perithecia usually are developing simultaneously and in close proximity, the carbonaceous tissue comes to surround several fruiting bodies, forming the so-called "stroma." This stroma is composed primarily of bark cells and differs materially from true stromata of the fungi, which consist only of fungus hyphae. The term "pseudostroma" has been applied to structures of this kind.

Proper taxonomic consideration of the Trypetheliaceae demands a more accurate knowledge of their microscopic characters, particularly those of the so-called "stroma," than is now available. Detailed developmental studies are needed on additional species and cultural studies

should also be made. The family presents a mixture of primitive lichen characters (crustose thallus, few lichen acids, no accessory thallus structures) with more advanced fungal characters ("stromata," many-septate spores, ascogonia provided with trichogynes). Probably it is not a natural group, but we do not yet know how family and generic distinctions should be changed. Considering the status of present knowledge, the limited area, and the small number of species concerned, the Zahlbruckner (**1926**) system of family and generic delimitation has been followed here in the interest of conservative treatment at this time. As previously indicated, in this system the Trypetheliaceae include those stromatic pyrenocarp lichens whose perithecia open by an apical pore. Two easily distinguished genera occur in Mississippi: (1) *Trypethelium*, with hyaline 3- to many-septate spores; and (2) *Melanotheca*, with brown, 3-septate spores.

KEY TO THE MISSISSIPPI TRYPETHELIACEAE

A. Spores hyaline, 3- to many-septate.....TRYPETHELIUM B
  B. Spores 3-septate when mature.....C
    C. Pseudostromata brownish, convex, containing numerous perithecia.....1. *T. mastoideum*
    CC. Pseudostromata black, composed of coalesced perithecial walls.....2. *T. tropicum*
  BB. Spores 5–9-septate when mature.....D
    D. Pseudostromata greenish-yellow to brown.....3. *T. virens*
    DD. Pseudostromata blackish-brown to black.....4. *T. scorites*
AA. Spores brown, 3-septate.....MELANOTHECA E
  E. Spores 12–18 μ long, thallus yellowish, pseudostromata black.....5. *M. aggregata*
  EE. Spores 22–30 μ long, parts of thallus and pseudostromata usually reddish or reddish-brown.....6. *M. cruenta*

1. TRYPETHELIUM MASTOIDEUM Ach., Lich. Univ. 307. 1810.
Bathelium mastoideum Ach., Method. Lich. 111. pl. 8, fig. 3. 1803.
Trypethelium Féei Meissn. apud. Fée, in Ann. Sci. Nat. **23**: 442. pl. 14, fig. 2. 1831.
Trypethelium scoria Nyl., Expos. Synopt. Pyrenocarp. 74. 1858 (excl. syn.).
Trypethelium carolinianum Tuck., in Amer. Jour. Arts & Sci. II. **25**: 429. 1858.

Thallus very thin (*Fagus, Liriodendron*) to well developed (*Quercus*), smooth, extending up to 9 or more cm in diameter, greenish-gray, yellow-brown, to brownish, bounded by a black margin (especially on *Liriodendron*, not always evident on other substrates); cortex amor-

phous, up to 30 μ thick; gonidia *Trentepohlia*, in scattered layers 15–30 μ thick; pseudostromata ashy to light brown (*Acer*), dark brown (*Liriodendron, Quercus*), sometimes blackish when mature, appearing as irregular masses of varying size, from 1 mm in diameter to structures 9 mm long × 3 mm wide, up to 0.3–0.5 mm high, occasionally containing altered bark cells; perithecia 0.3–0.4 mm high × 0.2–0.4 mm in diameter, with carbonaceous walls 10–30 μ thick, often with distinct pseudoparenchymatic area near the outer portion of the wall; ostiole minute; asci 75–105 μ long × 12–18 μ wide; paraphyses thin, unbranched; spores hyaline, 3-septate, 18–28 μ long × 6–9 μ wide (see FIG. 1).

Collected on: *Quercus nigra, Quercus phellos, Quercus* sp., *Acer rubrum, Acer* sp., *Liriodendron tulipifera, Fagus* sp., *Carya* sp., *Fraxinus americana, Prunus serotina, Magnolia macrophylla, Magnolia virginiana.*

Collected from: Adams, Amite, Carroll, Clarke, Forrest, Franklin, Greene, Harrison, Holmes, Jackson, Jefferson, Jones, Kemper, Lamar, Lauderdale, Lowndes, Marion, Neshoba, Pearl River, Perry, Pike, Rankin, Stone, Warren, Wayne, and Wilkinson Counties.

Specimens cited: G. T. Johnson, Nos. 331, 333, 337, 342, 343, 350, 354, 366, 1096, 1099, 1103, 1114, 1127, 1130, 1131, 1141, 1142, 1143, 1145, 1150, 1157, 1158, 1161, 1162, 1164, 1167, 1170, 1177, 1183, 1187, 1188, 1190, 1196, 1199, 1206, 1207, 1212, 1220, 1237, 1240, 1245; G. T. Johnson and H. N. Andrews, Nos. 1110, 1112, 1117, 1120, 2258, 2260, 2263, 2264, 2366, 2435, 2459, 2464, 2470, 2488, 2529, 2532, 2556, 2588, 2644, 2646, 2808, 2882, 3005, 3007.

2. TRYPETHELIUM TROPICUM (Ach.) Müll. Arg., in Engler's Bot. Jahr. **6**: 393. 1885.

Verrucaria tropica Ach., Lich. Univ. 278. 1810.

Sagedia tropica (Ach.) Mass., Ricerch. Auton. Lich. 161. fig. 161. 1852.

Pyrenula tropica (Ach.) Trevis., Spighe e Paglie 17. 1853.

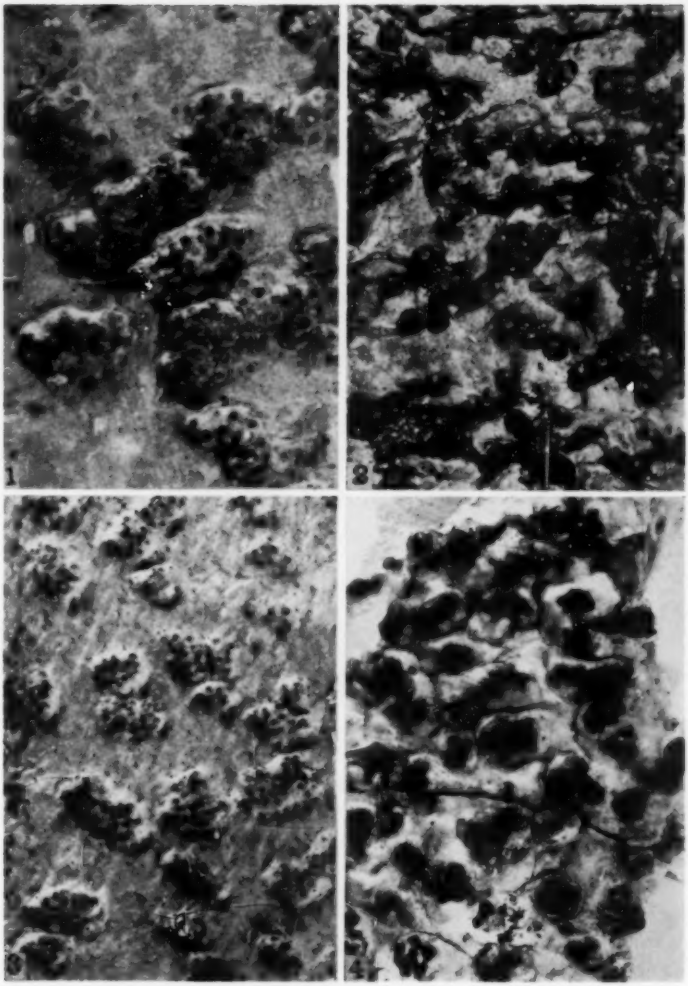
Spermatodium tropicum (Ach.) Trevis., Conspect. Verruc. 11. 1860.

Pseudopyrenula tropica (Ach.) Müll. Arg., in Flora **66**: 248. 1883.

Verrucaria Gaudichaudii Fée, Essai Cryptog. Écorc. Officin. 87. pl. 22, fig. 4. 1824.

Pyrenula Gaudichaudii (Fée) Pers. apud Freycin., Voyage Uranie et Physic. Bot. **28**: 480. fig. 190. 1895.

Thallus thin to somewhat raised above the bark, not continuous on older substrates where pustules 0.1 mm high may be formed, varying from small patches to areas 7 cm high × 14 cm wide, greenish-yellow (*Acer*), yellow (*Magnolia*), to orange-yellow (*Fagus*, older *Magnolia*),



FIGS. 1–4. Species of *Trypethelium*. FIG. 1. *T. mastoideum* Ach., H. W. Ravenel No. 275c; FIG. 2. *T. tropicum* (Ach.) Müll. Arg., Johnson and Andrews No. 2531; FIG. 3. *T. virens* Tuck., G. T. Johnson No. 1172; FIG. 4. *T. scorites* (Tuck.) Nyl., Curtis No. 256 (CO-TYPE). Approximate magnifications: FIGS. 1 and 2, × 10; FIG. 3, × 7; FIG. 4, × 5.

sometimes bounded by a jet black margin 0.5–1.5 mm wide, cortex amorphous, 18–45 μ thick; gonidia *Trentepohlia*, in layers 15–30 μ thick; pseudostromata more or less irregularly shaped, black in color, up to 5 mm long × 2 mm wide, extending above the thallus, composed of coalesced perithecia; perithecia usually aggregated in pseudostromata (sometimes solitary), 0.3–0.6 mm high, 0.35–0.60 mm in diameter, with carbonaceous walls up to 30–35 μ thick, with flattened top, often with slightly pruinose margin around the minute ostiole; hypothecium 15–30 μ high; asci 120–130 μ long × 12–15 μ wide; paraphyses thin, unbranched; spores hyaline, 3-septate, 20–26 μ long × 6–8 μ wide (see FIG. 2).

The pseudostroma of *T. tropicum* differs from the other Mississippi Trypetheliaceae because it is formed by the union of the carbonaceous walls of several perithecia that developed in close proximity and it contains few cells from the bark on which it grows. Since perithecia may occur singly as well as in groups this species has sometimes been referred to the Pyrenulaceae. Its eventual disposition may be an open question, but the best temporary assignment is in the Trypetheliaceae where most indices and manuals list it at the present time.

Collected on: *Acer rubrum*, *Acer* sp., *Quercus* sp., *Magnolia macrophylla*, *Magnolia virginiana*, *Ilex* sp., *Fagus* sp., *Fraxinus americana*.

Collected from: Clarke, Harrison, Hinds, Jackson, Jefferson, Jones, Lamar, Noxubee, Pearl River, Perry, Pike, Stone, and Wayne Counties.

Specimens cited: G. T. Johnson, Nos. 347, 1144, 1146, 1147, 1152, 1154, 1165, 1168, 1176, 1179, 1180, 1184, 1445, 1692; G. T. Johnson and H. N. Andrews, Nos. 1588, 2382, 2476, 2531, 2534, 2559, 2562, 2563, 2643, 2991, 3030.

3. TRYPETHELIUM VIRENS Tuck. apud Darl., Fl. Cestr., ed. 3. 453. 1853.

Trypethelium eluteriae Sprengl. var. *virens* (Tuck.) Trevis., in Flora **44**: 20. 1861.

Thallus thin, smooth, extending up to 12 cm in diameter, greenish (*Quercus*), yellow-brown to orange-brown (*Ilex*); cortex variable, 45–90 μ thick, often containing bark cells; gonidia *Trentepohlia*, occurring in layers 15–23 μ thick; pseudostromata up to 7 mm in length or width, slightly elevated, more or less concolorous with the thallus (especially when young) but lightening or darkening in color with age, with outer layer of bark cells (up to 55 μ thick), with perithecial initials forming below this layer, raising the bark layer as the perithecia develop, sometimes altering the bark cells to a blackish color; perithecia 0.4–0.6 mm high × 0.2–0.5 mm in diameter, with carbonaceous wall 30–50 μ

thick; hypothecium up to 30 μ in height; ostioles seen from above as black dots 0.1 mm in diameter; asci 100–125 μ high × 15–17 μ wide; paraphyses thin, unbranched; spores hyaline, 7–9-septate, 38–52 μ long × 7–10 μ wide (see FIG. 3).

Collected on: *Ilex opaca, Ilex decidua, Ilex* sp., *Quercus* sp., *Myrica cerifera, Celtis* sp., *Prunus serotina, Nyssa sylvatica.*

Collected from: Adams, Amite, De Soto, Forrest, Hinds, Jackson, Jefferson, Kemper, Lamar, Lowndes, Marion, Monroe, Montgomery, Newton, Oktibbeha, Pearl River, Perry, Pike, Rankin, Stone, Webster, and Wilkinson Counties.

Specimens cited: G. T. Johnson, Nos. 1006, 1010, 1013, 1015, 1048, 1049, 1050, 1053, 1054, 1055, 1056, 1057, 1059, 1092, 1109, 1111, 1115, 1116, 1119, 1129, 1133, 1134, 1135, 1136, 1172, 1175, 1192, 1194, 1201, 1209, 1211, 1215, 1217, 1218, 1227, 1695; G. T. Johnson and H. N. Andrews, Nos. 2379, 2386, 2516, 2642, 2645, 2715, 2777, 2931, 3008, 3029, 3373, 3375.

4. TRYPETHELIUM SCORITES (Tuck.) Nyl., in Ann. Sci. Nat. Bot. IV. **20**: 259. 1863.

Verrucaria scorites Tuck., *in herb.*

"*Verrucaria scorites* Tuck. est *Trypethelium* affine. In *Trypethelio scorite* (Tuck.) thallus macula pallida indicatus, apothecia in verrucis stromaticus fuscescentibus irregulariter rotundatis vel nonnihil difformibus (latitl. circiter 1 millim) convexulis, intus albidis, peritheciis nigris, ostiolis extus vix visibilibus; sporae 8^{nae} incolores oblongae 6–8-loculares (vel 5–7 septatae), longit. 42–52 μ, crassit. 12–17 μ. Lectum fuit in Mississippi, ad corticem, ex hb. Tuckerman. *Trypethelium virens* Tuck., Nyl., p. 77, quoque rite sicut species distincta considerandum sit" (see FIG. 4).

Nylander's (**1863**) type description is quoted above since this species was not recollected in the field. Although Mississippi is cited as the type locality the only authentic material in the Tuckerman Herbarium was collected in North Carolina. The specimen is illustrated in FIG. 4, but due to its fragmentary nature no microscopic sections were prepared for this study. Additional field work should be carried out before *T. scorites* is relegated to synonymy, but the writer suggests that the specimen illustrated is a mature *T. virens* where secondary growth (with resultant additional carbonization) has taken place in the perithecia and pseudostromata. Fink (**1935**) cites this species as occurring in North Carolina, Florida, Alabama, and Mississippi; however, the few specimens with somewhat blackish pseudostromata that the writer has seen from these localities are referable to *T. virens.*

5. MELANOTHECA AGGREGATA (Fée) Müll. Arg., in Mém. Soc. Phys. Hist. Nat. Genève **30** (3) : 18. 1888.

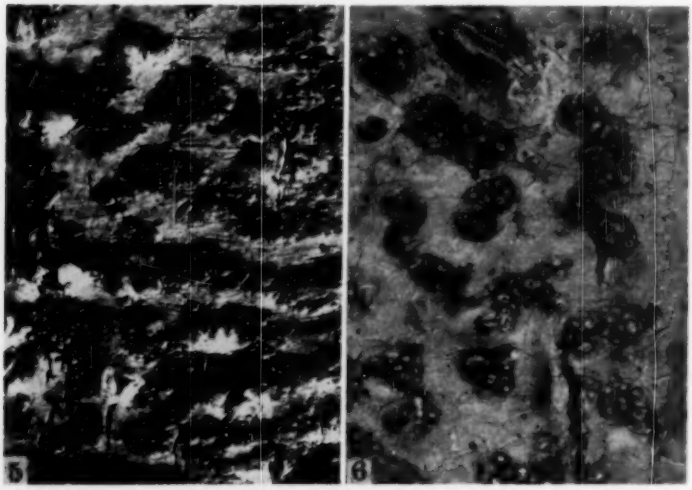
Verrucaria aggregata Fée, Essai Crypt. Écorc. Officin. 91. 1824.

Pyrenula aggregata (Fée) Fée, Suppl. Essai Crypt. Écorc. Officin. 61. 1837.

Spermatodium aggregatum (Fée) Trevis., Conspect. Verruc. 10. 1860.

Thallus thin, smooth, extending up to 12 cm in diameter, gray (*Liriodendron*), yellow-gray (*Symplocos*), to yellow-orange (*Ilex, Magnolia*), with jet black pseudostromata; cortex amorphous, 45–60 μ in height; gonidia *Trentepohlia*, in layers 15–25(45) μ thick, occasionally occurring in alternate layers with rows of bark cells; pseudostromata black, more or less irregular in shape, up to 5 mm long × 3 mm wide in younger specimens, later united in larger aggregates, only slightly raised above the thallus surface; perithecia 0.18–0.24 mm high × 0.28–0.30 mm in diameter, with carbonaceous wall 15–45 μ thick; hypothecium scant; ostiole minute, sometimes slightly depressed; asci 30–60 μ long × 8–10 μ wide; paraphyses thin, unbranched; spores brown, 3-septate, 15–18 μ long × 6–9 μ wide (see FIG. 5).

Collected on: *Ilex opaca, Liriodendron tulipifera, Magnolia* sp., *Nyssa sylvatica, Symplocos tinctoria.*



FIGS. 5–6. Species of *Melanotheca*. FIG. 5. *M. aggregata* (Fée) Müll. Arg., Johnson and Andrews No. 2485; FIG. 6. *M. cruenta* (Mont.) Müll. Arg., G. T. Johnson No. 1122. Approximate magnifications: FIGS. 5 and 6, × 7.

Collected from: George, Jackson, Jones, Marion, Perry, and Stone Counties.

Specimens cited: G. T. Johnson, Nos. 1137, 1151; G. T. Johnson and H. N. Andrews, Nos. 2561, 2635, 2677, 2750, 2775.

6. MELANOTHECA CRUENTA (Mont.) Müll. Arg., in Engler's Bot. Jahr. **6**: 397. 1885.

Trypethelium cruentum Mont., in Ann. Sci. Nat. Bot. II. **8**: 537. 1837.

Stromatothelium cruentum (Mont.) Trevis., in Flora **44**: 20. 1861.

Pyrenula cruenta (Mont.) Wainio, Étud. Lich. Brésil **2**: 199. 1890.

Thallus thin, smooth, extending 2, 4, or 6 cm in diameter, greenish, yellow-orange, or reddish-orange; cortex amorphous, 60–75 μ thick; gonidia *Trentepohlia*, in layers 15–30 μ thick; pseudostromata more or less irregular in shape, between 2 and 10 mm in length or width, dark red in color (especially when young; in older specimens the pigment may diffuse over the surface of the thallus giving the latter a reddish tinge, and the pseudostromata may then appear black with red margins); perithecia 0.25–0.60 mm high × 0.4–0.8 mm in diameter, sometimes extending one-third of their height above the thallus surface, with carbonaceous walls 50–130 μ thick; ostiole large, depressed and surrounded by whitish margin in older specimens; asci 130–150 μ high × 21–30 μ wide; paraphyses thin, unbranched; spores brown, 3-septate, 14–30 μ long × 9–16 μ wide (see FIG. 6).

Collected on: *Ilex opaca*, *Ilex* sp., *Quercus nigra*, *Quercus* sp., *Liriodendron tulipifera*, *Acer rubrum*, *Nyssa sylvatica*, *Carya* sp., *Prunus serotina*, *Fraxinus americana*.

Collected from: Adams, Amite, De Soto, Harrison, Jefferson, Jones, Lamar, Perry, Pike, Stone, Wayne, and Wilkinson Counties.

Specimens cited: G. T. Johnson, Nos. 330, 340, 351, 1122, 1125, 1153, 1155, 1156, 1173, 1189, 1202, 1221, 1225, 1226, 1228, 1233, 1241, 1244; G. T. Johnson and H. N. Andrews, Nos. 2440, 2443, 2541, 2553, 2597, 3026, 3374.

SUMMARY

This work represents the first extensive collection of the Trypetheliaceae in the United States and is one of the few investigations of the group in which field studies have been made. Five species: (1) *Trypethelium mastoideum*, (2) *Trypethelium tropicum*, (3) *Trypethelium virens*, (4) *Melanotheca aggregata*, and (5) *Melanotheca cruenta* are now known to be relatively common in Mississippi. A sixth species,

Trypethelium scorites, which has previously been reported from this state, is considered a doubtful species. Data are presented on the morphology, geographical distribution within the state, and substrate range of the species treated.

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Nylander, W. 1863. Lichens, in Triana, J., and J. E. Planchon, Prodomus Florae Novo-Granatensis. Ann. Sci. Nat. Bot. IV. **20**: 228–279.

Smith, A. L. 1921. Lichens. xxviii + 464 pp. *135 figs.* Cambridge.

Zahlbruckner, A. 1926. Lichenes. Spezieller Teil, in Engler-Prantl, Die nat. Pflanzenfam. *2* Aufl. **8**: 61–263. *96 figs.*

STUDIES ON THE GENUS PHYCOMYCES [1]

C. R. Benjamin [2] and C. W. Hesseltine

(with 8 figures)

Phycomyces belongs in the family Mucoraceae and asexually is more closely related to *Mucor* than to any other genus. It has globose sporangia containing many sporangiospores and typically *Mucor*-like columellae. The extremely long sporangiophores are similar to those of *Mucor,* except that they rarely branch. *Phycomyces* clearly differs from *Mucor,* however, in its mode of production of zygospores. These, rather than being supported above the substratum by suspensors attached to special zygophores as is the case in *Mucor,* are formed at the surface of the substratum between tong-shaped suspensors. From the suspensors are produced numerous dichotomously-branched, darkened spines or protuberances which might indicate a resemblance to *Absidia.* However, *Absidia* has its zygospores scattered through the aerial portion of the colony and never shows tong-shaped suspensors. In having such suspensors *Phycomyces* resembles members of the family Pilobolaceae. In that family the suspensors are directly in contact with each other at the surface of the substratum and the zygospore wall is smooth or nearly so, just as in *Phycomyces.* In these respects there is a resemblance also to the zygospores of *Piptocephalis* and *Syncephalis.* However, in neither of these genera nor in the Pilobolaceae are there ornamentations on the suspensors. According to Cutter (**1942**), the nuclear pattern in *Phycomyces blakesleeanus* and *P. microsporus* is different from that of any of the other genera of the Mucorales which he studied. Recent cytological studies have also been made by Robinow (**1957**) and Harm (**1957**) on *Phycomyces blakesleeanus.* It is our opinion that *Phycomyces* is a genus without any known close relatives in the family Mucoraceae.

Phycomyces has been studied more extensively from a physiological standpoint than any other genus in the order, as evidenced by the pub-

[1] Contribution from the Northern Utilization Research and Development Division of the Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois.

[2] Present address: National Fungus Collections, Plant Industry Station, Beltsville, Maryland.

lication of nearly 150 papers. Because its members are totally deficient in thiamine, they have been used as thiamine assay organisms. The positively phototrophic sporangiophores are larger and longer than those of any other known fungi. Although carotene is known to occur in varying amounts throughout the order, the conditions and mechanisms of its formation have been studied most extensively in this genus.

PHYCOMYCES Kunze, in Kunze and Schmidt, Mykol. Hefte 2: 113. 1823.

Mycelium coarse and rapidly growing, branched, often yellow-colored; sporangiophores typically unbranched, erect, phototrophic, often several cm in length, bluish or metallic in color, bearing single, large sporangia; sporangia dark, globose or slightly flattened, deliquescent, columellate, many-spored; sporangiospores hyaline, smooth, one-celled; zygospores produced just above substrate, formed between tong shaped suspensors; suspensors bearing dark, stiff, dichotomously-branched projections which surround the zygospore.

TYPE: *P. nitens* Kunze.

The genus *Phycomyces* was erected by Kunze in 1823 and accepted by Fries in 1832. Fries treated it as a member of his "Ordo II. Mucorini" with two species, one of which, *P. splendens*, he described as new. His description of this species is so meager that it is impossible to know exactly what he had. Neither illustrations nor sizes, except the height of the fruiting structures, are given. The only subsequent report of the species was that by Bainier (**1903**). Our attempts to locate material of *P. splendens* have been unsuccessful. According to information kindly supplied by Dr. Rolf Santesson, Curator, Institute of Systematic Botany of the University of Uppsala, there is no specimen of the species "either in E. Fries' herbarium or in the Uppsala fungus collections on the whole." Dr. Santesson stated that "A great number of species published by Fries were evidently never kept by him for his herbarium."

The other species accepted by Fries (**1832**) was the type species *P. nitens*. According to Kunze this species was first described by Agardh in 1817 as *Ulva nitens*. Agardh's brief description indicated an organism with simple filaments of more than a hand's breadth that were shining and olive-green. No illustrations were given. It was found growing on the walls and timbers of an oil mill in Finland. Apparently Agardh mistook the greenish color of the sporangiophores for the green of an alga and, perhaps because of the tubular nature of the sporangiophores, assigned it to the genus *Ulva*. In 1823 Kunze found it growing

under similar circumstances, that is, in oil mills in Saxony and in the vicinity of Leipzig and Dresden. Kunze recognized it as a fungus and illustrated pyriform columellae with elliptical sporangiospores attached. His description is rather good, considering the date when the work was done, and obviously concerns *Phycomyces* as we interpret the genus today. Interestingly, Berkeley (**1860**) reported it growing on casks and on the walls in oil mills, but he renamed it *Mucor phycomyces*. Van Tieghem and Le Monnier (**1873**) redescribed *Phycomyces nitens*, giving excellent illustrations and adequate measurements of parts of the fungus. The genus became established with their work and has since been a recognized genus of the Mucoraceae by all students of the group.

KEY TO SPECIES OF PHYCOMYCES

1. Sporangiospores oval to elliptical 2
   2. Sporangiospores averaging 15–30 μ in length ..... *P. nitens*
   2′. Sporangiospores averaging 8–12 μ in length ..... *P. blakesleeanus*
1′. Sporangiospores globose ..... *P. microsporus*

PHYCOMYCES NITENS Kunze, in Kunze and Schmidt, Mykol. Hefte **2** 113, fig. 9 (a–e). 1823.

Mucor nitens Sprengel, Syst. Veg. **4**: 539. 1827.

Phycomyces splendens Fries, Syst. Myc. **3**: 308. 1832.

Byssus olivacea Winch, Trans. Nat. Hist. Soc. Northumberland, Durham and Newcastle-upon Tyne **2**: 121. 1838.

Periconia phycomyces Bonorden, Handb. Allg. Myc. p. 113. 1851.

Mucor phycomyces Berkeley, Outl. Brit. Fung., pp. 29, 407. 1860.

Mucor romanus Carnoy, Bull. Soc. Roy. Bot. Belg. **9**: 162. 1870.

Mucor violaceus Brefeld, Bot. Unters. Schimmelpilze **4**: 56, 92. 1881.

Phycomyces nitens (Agardh) Schroeter, in Cohn's Krypt.-Fl. Schlesien **3**(1): 209. 1886.

Phycomyces pirottianus Morini, Malpighia **10**: 89, figs. 8–12. 1896.

Phycomyces splendens Fries emend. Bainier, Bull. Soc. Mycol. France **19**: 166. 1903.

Colonies growing rapidly, covering media in Petri dishes in less than 4 days, filling the dishes and with sporangiophores often protruding from under the lids, growing to various heights depending on the substrate but typically at least 4–5 cm in height, showing sporangiophores and sporangia to the naked eye, Dark Ivy Green,[3] Deep Olive-Gray or Slate Olive (sometimes on malt agar to Olivaceous Black) in color, becoming browner after 1 month, showing abundant sterile mycelium just above

[3] Colors, where capitalized, are those used by Ridgway (**1912**).

the surface of the medium; colony reverse in yellow shades such as Deep Olive-Buff or Vetiver Green; odor absent at first, but characteristically sweet at 6 weeks; sporangiophores arising from substrate mycelium (often from slightly inflated, yellow-brown regions which resemble the trophocysts of *Pilobolus* and which measure 60–90 μ in diam.), tough, steel blue or blue-green in color, but yellowish-brown near their bases and becoming brown throughout their length by 6 weeks, minutely striate, with numerous droplets adhering to their smooth walls, constricted somewhat just below the sporangium, positively phototrophic, typically nonseptate, only abnormally branched and then the branch arising only a short distance beneath a sporangium, in some cases containing amorphous balls or masses of dark yellow-brown material, 35–150(200) μ in diam.; short sporangiophores in varying numbers are always interspersed with the typical long sporangiophores; sporangia formed within 48 hours and begin to form when the sporangiophores are still very short (2–4 mm), borne erect from the first, yellow or yellow-orange, becoming greenish-olive or dark greenish-olive in reflected light on maturing, black in transmitted light, globose to dorsiventrally flattened, smooth-walled, with walls of the larger sporangia deliquescing, 140–500 μ in diam.; shape of columellae highly variable but dependent upon size, in the smallest being applanate, then conical, campanulate, cylindrical and pyriform in the largest; columellae smooth, light brown in color, with granular contents, with collar, 60–290 × 50–200 μ, in the smallest about 50 μ in length and 80 μ in width, in some strains up to 340 × 230 μ; sporangiospores smooth, hyaline, with contents faintly yellow-orange (especially in the center), mostly oblong-elliptical to oval in shape but a few globose and a few nearly reniform, 10.5–30 × 6.5–17 μ, averaging over 15 μ in length, where globose, 10.5–19.5 μ in diam.; substrate mycelium without chlamydospores or oidia but showing swollen thin-walled regions in feeder-hyphae close to the main filaments—these regions are intercalary, oval to obovate, up to 250 × 150 μ in size, and may contain yellow droplets in cultures 6 weeks old or may be empty; substrate hyphae upon aging containing yellow droplets; zygospores forming at 15–20° C but not at 7° or 24° C on special media, located just above substrate between equal tong-shaped suspensors, black, globose, faintly tuberculate, fragile (breaking readily under cover slips), 250–350 μ in diam.; progametangia hyaline with yellow-orange contents, coiled at their bases and initially parallel to each other, then forced apart except at their tips—resembling a pair of tongs; suspensors equal, 150–200 μ in diam. next the zygospore, hyaline to faintly gray, light brown or even purple, smooth except for projections which arise next the zygospore, appearing empty or sometimes with yellow material, especially in the suspensors first forming projections; the "first-formed" projections about 7–10 in number, at first hyaline, then brown and finally black, nonseptate, as much as 5-times branched with branches ultimately end-

ing as sharp points, with branching both dichotomous and irregular, 25–35 μ in diam. at base and 150–450 μ in length, typically with one portion covering the zygospore and another standing upright; projections on second suspensor formed in same manner but later, at first hyaline, becoming gray to brown, nonseptate, irregularly branched and not as many times as are the "first-formed" projections on the other suspensor, approx. 25 μ in diam. at their bases, up to 200 μ in length; heterothallic.

TYPE: Since the original material upon which Kunze based his species is probably no longer in existence, the authors propose NRRL 1467 (Blakeslee's strain No. C 1013) as a NEOTYPE.

The preceding description of the asexual stage is based upon studies of *P. nitens*, especially NRRL 1467, grown on synthetic mucor agar (SMA) (Hesseltine, **1954**) at 25° C for 10 days. The description of the zygosporic stage is based on studies of NRRL 2444 × NRRL 2678 grown on potato-dextrose agar (PDA) (Hesseltine, **1950**) and incubated at 15° C for 11–12 days. The sources of the *P. nitens* cultures are presented in TABLE I.

Certain observations were also made regarding the growth of *P. nitens* on other media. On Czapek's solution agar (Haynes et al., **1955**) growth is slight and only a few sporangiophores are formed next the block of inoculum, but often the previously described "swollen" cells which appear on the feeder hyphae can be seen. On hay-extract agar growth and sporulation are moderate and essentially alike in various strains. On malt-extract agar (Haynes et al., **1955**) and on PDA, growth is essentially as described on SMA, with mature sporangia being produced in 48 hours at 25° C.

A characteristic of this species is the occurrence of short sporangiophores. These vary in number from strain to strain and also in height, which often is no more than 500 μ. They are sometimes septate, hyaline or faintly brown below, and smooth-walled. Their sporangia are globose, 40–180 μ in diam., and contain relatively few spores which are, however, of the size range of the spores in sporangia of the large sporangiophores. The sporangial wall is relatively persistent. The columellae within the sporangia of these small sporangiophores are globose, oval or applanate and can readily be seen in intact sporangia. In some cases the bases of the columellae resemble those seen in *Absidia* where an apophysis is present.

P. nitens has been variously interpreted. In addition to the early observations of Agardh and Kunze, Sprengel (**1827**) called the species *Mucor nitens* and referred to both Agardh's and Kunze's descriptions.

TABLE I

SOURCES OF PHYCOMYCES NITENS CULTURES

NRRL No.	Source*	Mating reaction
1467	Burgeff, as *P. theobromatus* → CBS → Blakeslee, 1929, as C1013	+
2444	G. W. Martin, collected on rabbit dung, Surrey, England	+
2445	G. W. Martin, from litter under beeches, England	+
2453	Burgeff → CBS	+
2678	From Kominami → Nagao Institute 1161	−
2679	D. A. Gibson, from refuse on warehouse floor, Hull, England → CMI 44751	−
A-3622	CBS	+
A-5920	CBS	−
A-6297	W. H. Wilkins → NCTC 2895 → CMI 21999	−
A-6298	G. M. Waterhouse, from decaying wasp nest, North Ireland → CMI 31598	+
A-6302	D. J. Parry, from coconut meal, Bristol, England → CMI 51077	+
A-6322	Burgeff, *P. theobromatus* → CBS → CLMR → HUT → IFO 5694	+
A-6323	Burgeff, *P. theobromatus* → CBS → CLMR → HUT → IFO 5695	+
A-6372	A. E. de Areã Leão, as 2109	+
A-6419	Tsubaki → Nagao Institute 1160	−
A-6492	R. Cain, as No. 31515, isolated from bark of *Tsuga*, Ontario, Canada	+
A-6502	J. Nicot 497	+
A-7035	G. W. Martin, from dead wood, Urbana, Illinois	+
A-7211	M. Bound, from beans on sawdust, England → CMI 63923	+
A-7595	C. T. Rogerson, from dung in gypsum mine, Blue Rapids, Kansas	+
A-7703	R. K. Benjamin, No. 538, from mouse dung, San Gabriel Mountains, California	+
A-7704	R. K. Benjamin, No. 539, from mouse dung, Claremont, California	+
A-7707	R. K. Benjamin, No. 577, from mouse dung, San Gabriel Mountains, California	+
A-7710	R. K. Benjamin, No. 608, from mouse dung, San Gabriel Mountains, California	+
A-7711	R. K. Benjamin, No. 608, from mouse dung, San Gabriel Mountains, California	+

* CBS = Centraalbureau voor Schimmelcultures, Baarn, Holland.
CMI = Commonwealth Mycological Institute, Kew, England.
IFO = Institute for Fermentation, Osaka, Japan.
CLMR = The Central Laboratory, South Manchuria Railway Co., Dairen, Manchuria.
HUT = Faculty of Technology, Hiroshima University, Japan.
NCTC = National Collection of Type Cultures, England.

Winch (**1838**) introduced an apparent synonym, *Byssus olivacea*, when he described an organism found on the walls of an oil cellar in Newcastle as having long, simple filaments of an olive-green color. Fries (**1832**) described *Phycomyces splendens* inadequately, as mentioned previously. Bainier (**1903**) redescribed it without illustrations, but from his description it would appear to be *P. nitens*, a view also held by Schroeter (**1886**), Fischer (**1892**) and Naumov (**1939**). Zycha (**1935**) recognized *P. splendens*, but he only quoted Bainier (**1903**) and did not actually study any material.

Bonorden (**1851**) transferred *Phycomyces nitens* to the genus *Periconia*, making the new combination *Periconia phycomyces* and listing *P. nitens* Kunze as a synonym. Berkeley (**1860**) described the species as *Mucor phycomyces*, in turn citing Kunze's *P. nitens* as a synonym. Van Tieghem and Le Monnier (**1873**) called attention to another synonym of *P. nitens*, described as *Mucor romanus* Carnoy, studied from human feces. The fungus had simple sporangiophores, 6 to 9 cm in height, which were colored. The sporangia were large and at first yellow, then dark. The columellae were shown with all the variations encountered in our studies of the species. The sporangiospores were of the shape and size of *P. nitens*, and we concur in the conclusion of Van Tieghem and Le Monnier that *M. romanus* is a synonym of it. In at least two places in his writings, Brefeld (**1881**) mentioned the name *Mucor violaceus*, which he used in preference to *P. nitens*. Schroeter (**1886**) apparently made the combination *P. nitens* (Agardh) Schroeter. The species is so cited in his treatment of the genus in Cohn's Kryptogamen Flora.

Morini (**1896**) described a low-growing form isolated from horse dung. Its sporangiospores were elliptical and pale yellow, but were smaller than previously described, measuring only 12–18 μ in length. Since the strain apparently was distinct, Morini described it as a new species, *Phycomyces pirottianus*. However, we have found in our study a strain which definitely fits the species concept of *P. pirottianus*, but which also shows the strongest reaction of any of our strains of *P. nitens* when mated with a larger-spored strain of the opposite mating type. We therefore have extended the spore-size range of *P. nitens* to include such forms.

PHYCOMYCES BLAKESLEEANUS Burgeff, Flora, New Series, **18**: 42 (Allgemeine Botanische Zeitung **118**: 42), figs. 1–2. 1925.

Colonies growing rapidly, covering media in Petri dishes in less than 5 days, filling the dishes and with sporangiophores usually protrud-

ing from under the lids, growing to various heights depending on the substrate, typically at least 4–5 cm in height, showing sporangiophores and sporangia to the naked eye, Dark Ivy Green to Deep Olive-Gray in color, with varying amounts of sterile mycelium just above the surface of the medium; colony reverse in moderately bright yellow-orange shades such as Deep Colonial Buff, Buff-Yellow and Apricot Yellow, with the color often unevenly distributed; odor faint and indefinite; sporangiophores arising from substrate mycelium, yellowish-brown at their bases, becoming greenish-gray to steel-blue further toward the apex, turning yellowish-brown upon aging, narrowed just below the sporangium, typically nonseptate and unbranched except where in contact with Petri dish lid, with growing tips hyaline to faintly yellowish and positively phototrophic, often with numerous adhering droplets, often appearing empty or with yellow droplets inside, generally 50–150 μ—rarely over 200 μ—in diam., commonly over 5 cm in length; short sporangiophores also present in more-or-less small numbers; sporangia formed after 3–4 days or more and generally after the sporangiophores have reached a length of 1 cm or more, hyaline to yellow when young, becoming dark olive-green to black on maturing, borne erect, deliquescing, globose to subglobose, many-spored, 150–520 μ in diam., occasionally larger; columellae conical, ovate, obovate, or even of a constricted-oblong shape where quite large, smooth, with or without collar, with hyaline to yellowish-brown contents, of various sizes to over 400 × 400 μ, but generally 150–300 × 100–225 μ; sporangiospores 1-celled, oval to oblong-elliptical, heavy-walled, smooth, with more-or-less granular contents and yellowish-orange pigment which often is concentrated centrally, 8–13 × 5–7.5 μ in size; substrate mycelium much branched, granular, often showing large, thin-walled terminal or intercalary cells; zygospores produced readily between compatible strains at room temperature or below on ordinary substrates, formed between tong-shaped suspensors at the surface of the substratum, black, globose, faintly roughened, readily crushed under a cover glass, mostly 400–500 μ in diam.; progametangia hyaline with yellow-orange contents, coiled at their bases and becoming tong-shaped; suspensors equal, commonly 150–200 μ in diam. next the zygospore, hyaline to light brown at their bases and progressively darker to black next the zygospore, smooth except for appendages near zygospore, usually containing yellow-orange droplets; appendages formed on both suspensors of a zygospore, at first hyaline but soon becoming black, about 6–10 in number, mostly nonseptate, 3–5 times branched with branching appearing dichotomous when young but irregular when mature and with ultimate branches distally somewhat pointed, 25–50 μ in diam. at their bases, commonly with one primary branch extending over the zygospore and the other upright; heterothallic.

TYPE: Burgeff's "Claussen (+)" strain.

The preceding description of the asexual stage is based upon studies of *P. blakesleeanus*, especially NRRL 1464 and NRRL 1465, grown on SMA at 25° C for 13 days. The description of the zygosporic stage is based on studies of NRRL 1554 × NRRL 1555 grown on PDA and incubated at 15° C for 17 days. Type material was not located.

The strains of *P. blakesleeanus* which we have studied conform to the description of the species given by Burgeff (**1925**) except for the slightly smaller sizes of the asexual structures of our strains. The species differs rather markedly from *P. nitens* by having larger zygospores and smaller sporangiospores. In addition, sporangial formation requires longer incubation and the columellae are often more generally oval than are those of *P. nitens*. Contrary to the case in *P. nitens*, *P. blakesleeanus* forms zygospores at 25–26° C on a variety of media. However, a larger number forms and more reach maturity at temperatures of 15–20° C.

The habitat of the early-isolated strains of *P. blakesleeanus* is unknown, according to Burgeff (**1928**) and Zycha (**1935**). NRRL 1464 (Blakeslee's No. C 1015) was obtained by Dr. Blakeslee from the Centraalbureau voor Schimmelcultures at Baarn. NRRL 1465 (Blakeslee's No. C 1041) was isolated in 1903 from rabbit dung. NRRL 1554 and NRRL 1555 might well have originally been Blakeslee's cultures, but were obtained for the NRRL collection in 1940 from L. H. Leonian. Of the 50 strains of *P. blakesleeanus* assembled for the present study there are only 12 for which we have isolation data. Eleven originated from dung and 1 from spore material of *Phallus impudicus* which had been sown on agar.

P. blakesleeanus has been called *P. nitens* by numerous workers. It was not recognized as a distinct species until Burgeff compared a strain received in 1921 with others in his possession. He found that the new strain agreed with the description of *P. nitens* as given by Van Tieghem and Le Monnier while others differed in several respects. These latter forms, which were fairly widely distributed in culture, were placed in his species *P. blakesleeanus*. Citations of work done on *P. blakesleeanus* under the name *P. nitens* are given by both Burgeff (**1925**) and Zycha (**1935**).

Earlier, Burgeff (**1912**, **1915**) had described two varieties of *"P. nitens"* called *"piloboloides"* and *"plicans."* These were actually derivatives of the "Claussen (+)" strain of *P. blakesleeanus*. Burgeff (**1928**) stated that both varieties were heterocaryotically combined with *P. blakesleeanus*, that *"plicans"* was a labile form, and that *"piloboloides"*

was a stable one. The variety "*plicans*" was said to resemble *Rhizopus arrhizus*. The variety "*piloboloides*," represented by NRRL 2565 (Burgeff's 20– strain) and NRRL 2566 (Zycha's– strain), resembles *Pilobolus* in that its sporangiophores have constricted apices subtended by inflated areas. Harm (**1957**) included two strains of this variety in her rather exhaustive study on *P. blakesleeanus*.

PHYCOMYCES MICROSPORUS Van Tieghem, Ann. Sci. Nat., Ser. 6, 1: 64. 1875.

Mycelium abundant, growing rapidly, producing a number of fruiting filaments in 5–6 days; sporangiophores simple, erect, as in *P. nitens* showing at their bases 1–2 sterile appendages like fingers of a glove, or ramified in the fashion of rootlets, cuticularized, becoming colored early from base to top in greenish-brown with metallic reflections more-or-less marked, sometimes very dark and completely opaque at their bases, with octahedral crystals floating in the cellular sap, 4–5 cm in height; sporangia yellowish when young, becoming black, with membranes roughened with calcium oxalate crystals, totally diffluent in water, columellate, many-spored, about ½ as large as in *P. nitens;* columellae with membrane neither cuticularized nor colored, practically cylindrical, a little constricted in the middle, filled with yellowish-orange granular protoplasm; sporangiospores held together by an interstitial hyaline substance, spherical, a little irregular ["inégales"], individually uncolored, *en masse* lightly yellowish, averaging 8 μ in diam.; zygospores spherical, laterally attached to arched suspensors, black at maturity, about half the size of those of *P. nitens,* measuring about 125 μ in diam.; suspensors dark-colored, arched, bearing in the vicinity of attachment to the zygospore black appendages or spines which are about 3 in number per suspensor and which probably are branched.

TYPE: Unknown.

The preceding description is based on Van Tieghem's original one. We have seen no representatives of this species. Van Tieghem based his description on his studies of one zygospore which had germinated forming a sporangium on horse dung, and on its progeny cultivated on boiled dung and sections of orange.

Van Tieghem stated that *Phycomyces microsporus* was clearly distinguished from *P. nitens,* under similar nutritive conditions, by the following characteristics: its fruiting filaments were smaller and only half as tall, its sporangia were only half as large, its spores were very small, spherical and hyaline, and its zygospores were very small and bore only 3 branched spines from each side. In addition, *P. microsporus*

differs from *P. blakesleeanus* in having spherical sporangiospores and zygospores only about $\frac{1}{4}$ as large.

Both (+) and (−) strains of *P. microsporus* were reported by Christenberry (**1940**) as isolated from dung collected in North Carolina. Christenberry both described and illustrated the species as represented by the new strains. He described the sporangiospores as elliptical, 7.0–11.5 × 5.0–6.6 μ, with wall smooth and 1 μ thick. Such spores are entirely typical of *P. blakesleeanus*, whereas the spherical ones of the original description are not. Both Van Tieghem and Christenberry describe the zygospores as spherical and 125 μ in diameter, a size much smaller than is typical for *P. blakesleeanus*. The zygospore illustrated by Christenberry resembles ones which we have seen produced by *P. blakesleeanus* under conditions unfavorable for full development.

P. microsporus has not been completely accepted by mycologists. Fischer (**1892**) included the species in his treatment of the genus, but Schroeter (**1897**) did not. Fitzpatrick (**1930**) mentioned it as a recognized species. Zycha (**1935**) included the species, but stated that it evidently was only a stunted form of *P. blakeleeanus*. Naumov (**1939**) included it in his key to species, but made no reference to the species in his later work (**1954**). This partial rejection of the species may well be due to the rarity of reports of its occurrence. In our opinion, however, the original description would seem sufficient to distinguish the species, and in the absence of authentic material for study and of evidence for discarding the species, it should be maintained.

DOUBTFUL SPECIES

PHYCOMYCES SPINULOSUS Morini, Acad. Sci. Inst. Bologna, Ser. 6, Memorie **10**: 301, figs. 5–10. 1914.

This species was stated by Morini (**1914**) to be very similar to *Phycomyces nitens* in respect to its asexual characteristics. No formal description was given in Morini's article, and the only sizes mentioned were those of the sporangiophores, which were about 6–8$\frac{3}{4}$ cm in height. The species was erected primarily on the basis of its unusual zygospore type, which Morini said might be important in diminishing the phylogenetic gap between *Phycomyces* and *Mucor*. Morini's illustrations show equal, smooth, inflated suspensors bearing a subglobose zygospore, which is covered by short, bifid spines or projections of the zygospore wall.

In order to confirm Morini's observations, we attempted without success to obtain authentic material for study by writing the several

institutions listed by Ciferri (**1952**) as possessing Morini's types. If the described type of zygospore formation is constant and normal, *P. spinulosus* is truly distinct; otherwise, it probably is a synonym of *P. nitens*.

ZYGOSPORE FORMATION IN PHYCOMYCES NITENS

When various strains of *Phycomyces nitens* were secured for study, many were mated with each other at random on PDA, malt agar and

TABLE II

MATING REACTION OF STRAINS OF P. NITENS WHEN MATED WITH P. NITENS STRAIN NRRL 2444 (+) AND WITH P. BLAKESLEEANUS STRAIN NRRL 1555 (−) AT 20° C

NRRL Strain No.	NRRL 2444		NRRL 1555	
	Reaction	Number of fusions	Reaction	Number of fusions
2445	−		+	600+
2453	−		−	
2678	+	600+	−	
2679	+	500+	−	
A-5920	+	400	−	
A-6297	+	70+	−	
A-6298	−		+	250+
A-6302	−		+?	0
A-6322	−		+	90+
A-6323	−		+	500+
A-6419	+	undet.	−	
A-6492	−		−	
A-6502	−		−	
A-6736	−		+	60+
A-7035	−		+	400+
A-7211	−		+	0
A-7595	−		+	300+

+ = Mating reaction.
− = No reaction.

yeast extract agar in Petri dishes incubated at 25° C. No zygospore formation occurred and, in fact, not even a positive reaction such as the formation of thicker mycelium or an enhancement of yellow pigment appeared. Furthermore, when the *P. blakesleeanus* mating types, NRRL 1554 and 1555, were each mated with some of the *P. nitens* isolates, no reaction occurred. Other experiments involved attempts to mate *P. nitens* NRRL 1467 with all the other strains at 6–7° C on malt agar. Although growth was slow at this temperature, eventually a heavy vegetative growth occurred but zygospore formation did not.

Additional experiments involved other strains mated in various

combinations on SMA, PDA and YpSs (Emerson, **1941**) agar exposed to direct sunlight and with fluctuating temperatures. Various combinations of strains and various media were tried at 25° C in the hope that two strongly mating strains might be brought together to give a reaction, no matter how slight. We had noted earlier that corn steep liquor agar had been the best medium for mating-reactions in *P. blakesleeanus*. This medium had been tried at 25° C with *P. nitens* unsuccessfully. In our first successful experiment, the matings were made at 20° C in diffuse light in which NRRL 2444 was combined with certain strains

TABLE III

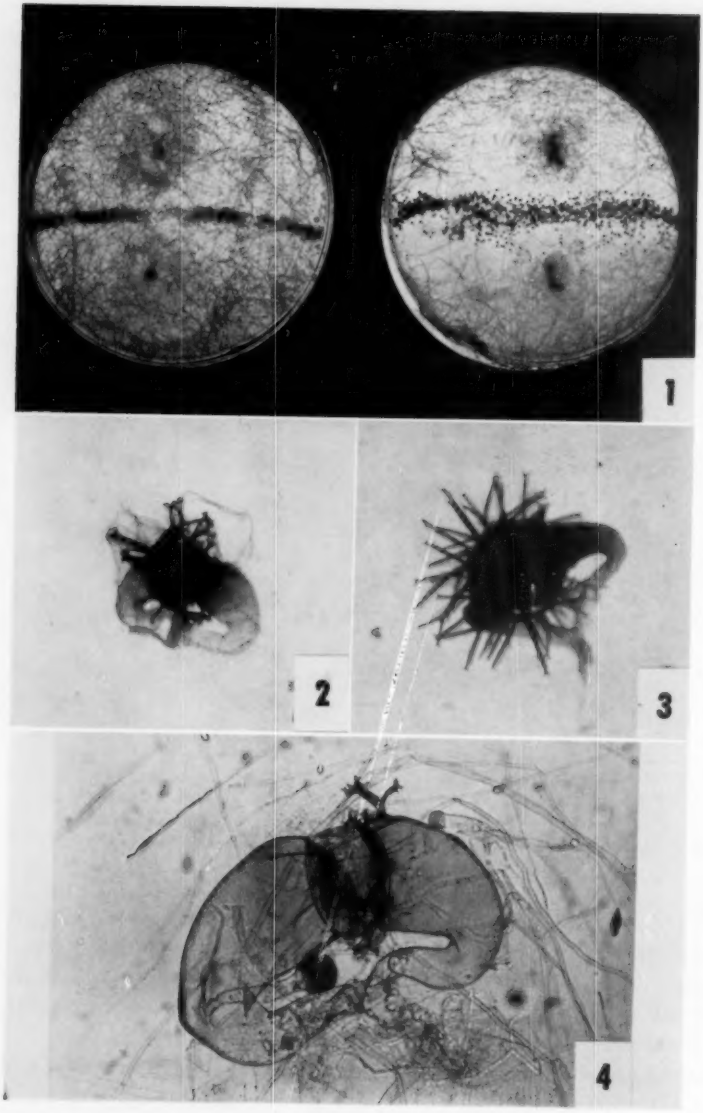
EFFECT OF VARIOUS MEDIA ON ZYGOSPORE FORMATION WITH P. NITENS STRAINS NRRL 2444 AND NRRL 2678 AT 20° C

Medium	Mating reaction	Average number of fusions
Corn steep liquor agar	+	500
PDA	+	450
Tomato-paste oatmeal agar*	+	225
YpSs agar	+	135
Corn meal agar	+	81
Glucose asparagine agar**	+	66
Oatmeal flakes in water agar	+	60
Czapek's solution agar	+	0.5
20% Czapek's solution agar	−	
Emerson's agar	−	
Hay agar	−	
Lima bean agar	−	
Malt agar	−	
SMA	−	
Starch agar	−	
Yeast extract agar	−	

* See Pridham, et al. (**1956–57**).
** See Barnett and Lilly (**1950**).

of *P. nitens* then available. At the same time *P. blakesleeanus* strain NRRL 1555 (−) was also mated in separate plates with the *P. nitens* strains on the same medium. In 3 days typical tong-shaped progametangia were formed between NRRL 2444 and NRRL 2678, A-5920, A-6297, NRRL 2679, and A-6419. In every other case negative results were obtained. The zygospores seen are described above under the *P. nitens* species description. In matings of *P. blakesleeanus* with *P. nitens* strains, progametangia were formed between the species. TABLE II shows the results of this experiment.

The numbers given in TABLE II and subsequent tables are counts of the number of fused progametangia at the stage of maturity when the progametangia have separated from each other to give the appearance



FIGS. 1–4.

of a pair of tongs and all later stages. The zygospores were arbitrarily considered to be mature when they were black in color and always surrounded by the branched appendages from the suspensors.

The final examination of these matings was made at 23 days and only in a relatively few cases were zygospores black in color indicating maturity. Some were seen in the mating of NRRL 2679 × 2444 and 2678 × 2444. A few black zygospores were also seen when NRRL 1555 was mated with A-6298 and A-7035. In three instances, NRRL 2453, A-6502 and A-6492, no reaction occurred.

After the achievement of zygospore formation, NRRL 2444 and 2678 were judged the best mating types and subsequent studies were made with these strains. Our next experiment involved the preparation of a variety of media upon which these two strains were mated at 20° C. Each mating was made in duplicate. The media used and the results obtained are shown in TABLE III.

From actual counts of the tong stage, the three best media for further work were corn steep liquor agar, PDA and tomato-paste oatmeal agar. However, in none of these were mature zygospores seen even though from 400 to 500 fusions in the tong stage occurred on PDA. In general, growth was good on all the media listed in TABLE III, except Czapek's solution agar, 20% Czapek's solution agar and starch agar. The counts of the number of fusions were made at 12–14 days and all plates were incubated at 20° C. Of particular interest was the fact that a number of progametangial fusions occurred on the one synthetic medium, glucose asparagine agar, but failed to occur on a similar synthetic medium, SMA. These media are very similar in composition but are quite different in ratios of glucose to asparagine and also in the amounts of these substances. Another experiment was run using SMA with modified glucose to asparagine ratios. It was found that the tong stage in zygospore formation occurred when 2 gm of glucose and 0.5 gm of asparagine were used. This combination is similar to that used in the glucose asparagine agar medium. All other combinations of glucose and asparagine gave negative results.

Our next experiments were made to determine as far as possible the

FIGS. 1–4. *Phycomyces nitens* (NRRL 2444 × NRRL 2678) and *P. blakesleeanus* (NRRL 1554 × NRRL 1555). 1. Petri dish matings grown on PDA for 10 days at 15° C, showing relative sizes of *P. nitens* (left) and *P. blakesleeanus* (right) zygospores which occur in lines across the centers of the plates, × ½. 2. Zygospore of *P. nitens*, × 30. 3. Zygospore of *P. blakesleeanus*, × 30. 4. Very young zygospore of *P. nitens*, showing appendages beginning to form on one suspensor, × 90.

range of growth asexually of strains NRRL 2444 and NRRL 2678 and the range of temperatures where zygospores are formed. All matings were made on corn steep liquor agar in duplicate plates and read at 14 days, except those at 7° C which were read at 38 days. The results are summarized in TABLE IV. Upon this medium the colonies at 32° C were restricted to only a few mm in diameter and no sporangia were formed. At 28° C sporangiophores were more numerous but were generally quite short. It is obvious from this experiment that matings must be made at 20° C or less, but not at 7° C. A temperature of 15° C would appear to be the best of those tested.

TABLE IV

MATINGS OF P. NITENS STRAINS NRRL 2444 × NRRL 2678 ON CORN STEEP LIQUOR AGAR AT VARIOUS TEMPERATURES

Temperature ° C	Growth	Mating reaction	Average number of fusions
37	−	−	
32	+	−	
28	+	−	
24	+	+	14
20	+	+	500
15	+	+	774
7	+	−	

Burkholder and McVeigh (**1940**), Robbins and Schmidt (**1945**), and more recently, Gladtke and Brucker (**1958**), have reported on pH effects. The last authors, working with single strains of *P. blakesleeanus,* attribute the increased acidity to a selective intensified cation—especially K-ion—uptake. In our experiment, to check the possible effect of pH of the medium on the mating reaction of *P. nitens,* the strains NRRL 2678 and NRRL 2444 were grown on agar media in Petri dishes and used to inoculate flasks. One hundred-ml amounts of potato dextrose broth were sterilized in 500-ml flasks. One flask in each series of three was adjusted to a desired pH level and discarded. The other two were then adjusted aseptically to this pH with either H_2SO_4 or KOH. The flasks were inoculated with a small block of mycelium of each strain and incubated at 20° C for 20 days. At this time the pHs of the flasks were determined, and the mycelial mats were removed and examined to determine the number of fusions and the number of mature zygospores. The exact number of fusions was very difficult to determine because of the abundant formation of sporangiophores and aerial mycelium. Floating the pads on water made counting easier and more exact. The results are summarized in TABLE V.

TABLE V

EFFECT OF pH ON P. NITENS MATINGS

Initial pH	Final pH	Estimated number of	
		Progametangial fusions	Mature zygospores
3	2.65	0	0
3	2.66	0	0
4	3.23	0	0
4	3.45	5	4
5	4.01	350	26
5	4.18	400	60
6	4.51	750	250
6	4.49	800	200
7	4.69	850	300
7	4.79	600	200
8	4.80	650	250
8	4.82	1000+	300
9	5.25	1000+	300
9	5.28	1000+	350
10	5.77	500	150
10	5.84	400	100

The results in TABLE V indicate that zygospores were formed over a wide pH range. At the lowest pH vegetative growth was restricted and colonies actually did not reach each other. Another difficulty was the tendency of certain blocks of the inoculum to settle to the bottom of the flask. In these cases mycelium did reach the surface and aerial growth resulted. It should be noted that the proportion of mature zygospores is very low at ρH 5. Particularly striking was the fact that at more favorable pH levels larger numbers of mature zygospores were encountered than had ever been seen on PDA plates. Only two factors were different between these experiments, no agar was present in the flask matings and the volume of medium to the surface area differed from that of the Petri dishes. The following experiment was then

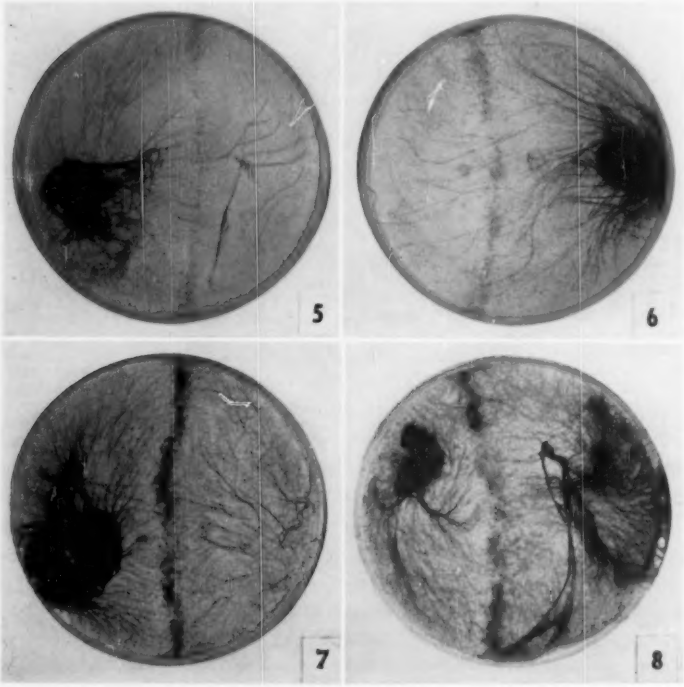
TABLE VI

EFFECT OF VARIOUS AMOUNTS OF POTATO DEXTROSE AGAR UPON ZYGOSPORE FORMATION IN P. NITENS

Ml of medium	Average number of fusions	Average number of mature zygospores
10	305	3.25
20	585	6.75
50	1600 (est.)	*
70	2260 (est.)	*

* Because of the number of fusions and amount of associated mycelium, no count of mature zygospores could be made.

made to determine if the volume of medium to surface area was significant. Potato dextrose agar was prepared in the usual fashion and added to deep Petri dishes ($10 \times 2\frac{1}{2}$ cm in size) in varying amounts. Each plate was then inoculated with NRRL 2678 × NRRL 2444. The plates were incubated at 20° C and read at 14 days. At each level



FIGS. 5–8. *P. nitens* (NRRL 2444 × NRRL 2678), showing influence of the quantity of a medium upon the number of zygospores formed. The mycelium in each dish has been pulled to one side to show the line of zygospores. 5. With 10 ml of medium. 6. With 20 ml of medium. 7. With 50 ml of medium. 8. With 70 ml of medium.

either 3 or 4 duplicate plates were used and the results are summarized in TABLE VI.

In TABLE VI it can be seen that an increase in the volume of the medium, surface area remaining constant, produces an increase in the number of progametangial fusions and a larger number of mature zygo-

spores. This point is graphically shown in FIGS. 5–8 and must be remembered when mature zygospores are wanted for study.

Having determined that the best temperature for mating work was either 15° or 20° when corn steep liquor agar was used, we studied other excellent media, including a synthetic one, to determine at what temperature and on which medium were produced the most fusions and mature zygospores. NRRL 2678 and NRRL 2444 were again mated as previously described on approximately the same amounts of appropriate media. Each condition was set up in triplicate. The results, summarized in TABLE VII, were read in the usual fashion at 18 days.

TABLE VII

AVERAGE NUMBER OF FUSIONS OF PROGAMETANGIA AND MATURE ZYGOSPORES OBTAINED ON VARIOUS MEDIA AT 15° AND 20° C

Medium	15° C		20° C	
	Number of fusions	Mature zygospores	Number of fusions	Mature zygospores
Glucose asparagine agar	84.3	5.3	136.6	4.3
Potato dextrose agar	453.3	90.6	278.3	29.0
Tomato-paste oatmeal agar	453.3	168.0	67.3	13.6

It can be seen that in every case the average number of progametangial fusions and the average number of mature zygospores were greater at 15° C than at 20° C, except for the number of fusions at 15° C on the glucose asparagine agar.

SUMMARY

The genus *Phycomyces* has been reviewed and revised taxonomically. It has been found to comprise the three species, *P. nitens* Kunze, *P. blakesleeanus* Burgeff, and *P. microsporus* Van Tieghem. *P. spinulosus* Morini is treated as a doubtful species, and *P. pirottianus* is reduced to synonymy with *P. nitens*. Descriptions and synonymy are presented, as are the results of numerous mating experiments with strains of *P. nitens* subjected to various experimental conditions.

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THE DICTYOCHLAMYDOSPORE OF PEYRONELLAEA GLOMERATA (CORDA) GOIDANICH EX TOGLIANI CONTRASTED WITH THE DICTYOPOROSPORE OF ALTERNARIA TENUIS AUCT.

GEORGE M. LUEDEMANN

(WITH 18 FIGURES)

The genus *Peyronellaea* is a segregate of the large genus *Phoma* of the Fungi Imperfecti. In addition to a typical pycnidium and pycnidiospores, catenate dictyospores are found in the developmental cycle. Species belonging in this genus have been reported in the literature as pathogens of both plants (**3**, **6**, **7**, **9**, **10**, **11**, **12**, **13**, **14**, **17**, **19**, **22**, **23**, **26**, **29**, **31**) and animals (**1**, **4**, **13**, **25**), but their role is probably that of secondary and not primary invaders. In the present study isolates of *Peyronellaea glomerata* were consistently obtained from senescent and dead leaves of a variety of deciduous plants.

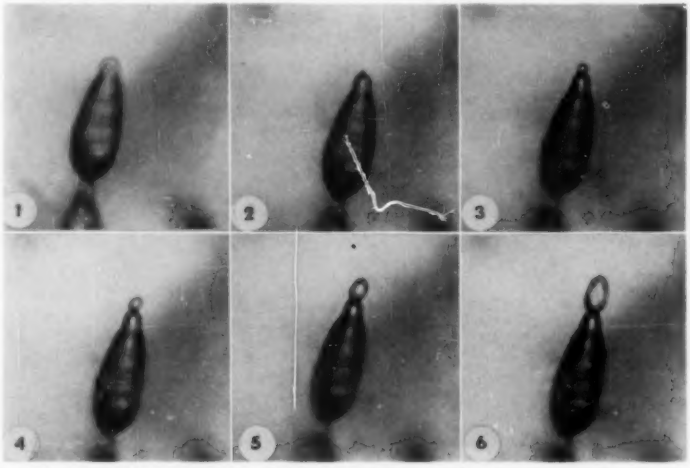
Organisms with both phomoid and dictyosporic stages have been placed in numerous genera. The dictyosporic form has been placed in such genera as *Coniothecium* (**19**, **22**) and *Alternaria* (**1**, **11**, **24**), while the pycnidial stage has been placed in *Phoma* (**23**, **28**, **31**), *Ascochyta* (**7**, **10**), *Aposphaeria* (**16**), *Coniothyrium* (**8**), and *Phyllosticta* (**9**). Goidanich (**13**) established the genus *Peyronellaea* for those forms with a phomoid pycnidial stage accompanied by a dictyosporic stage. Goidanich did not include a formal Latin diagnosis for the genus. In 1952 Togliani (**30**) validated this genus by publishing a formal Latin diagnosis and designated *Phoma glomerata* (Corda) Wollenweber and Hochapfel as the type species for the genus.

Phoma glomerata was originally described as a *Coniothyrium* by Corda (**8**). Wollenweber and Hochapfel (**31**) transferred it to the genus *Phoma* and added the description for the dictyospores. An extensive synonymy was listed for *Phoma glomerata* by Wollenweber and Hochapfel. The writer, from cultural studies [1] of organisms obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland,

[1] Part of a doctorate thesis entitled "A Study in the Genus *Peyronellaea*" submitted to Syracuse University in 1957 by the author.

would include in the synonymy of *Peyronellaea glomerata* the species *P. conidiogena* Schnegg (Benham's isolate) (**4, 28**), *P. hominis* Agostini and Tredici (**1, 25**), and *P. fumaginoides* Peyronel (Sibilia's isolate) (**11**).

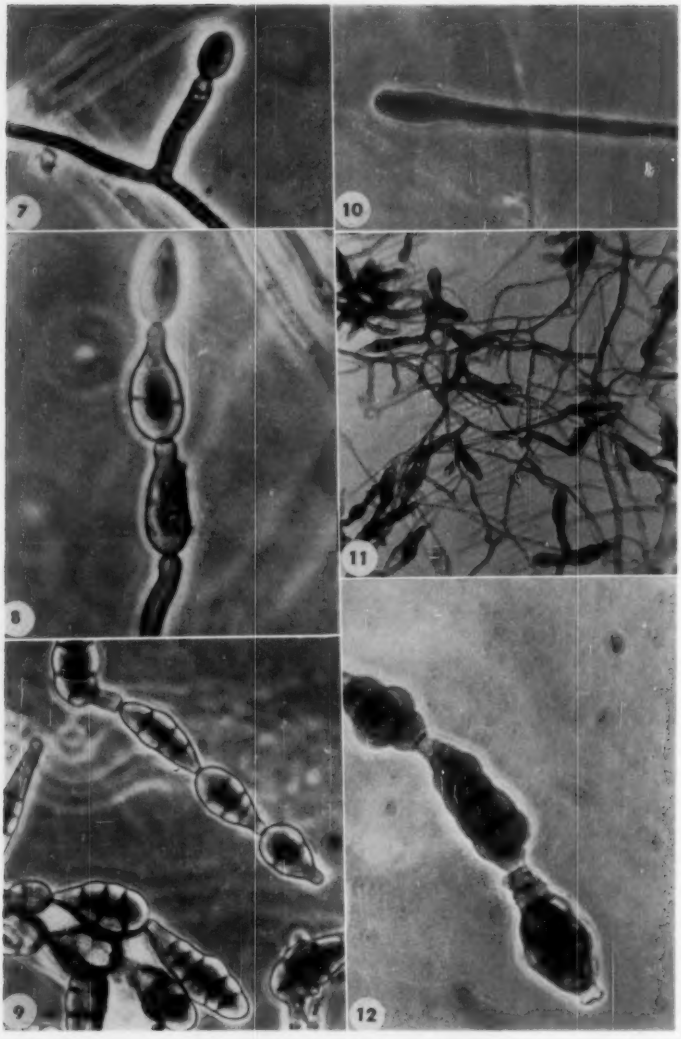
In consideration of the number of species placed in synonymy and the general lack of differentiating characteristics in species descriptions, it is probable that only two well defined morphological species exist. These are *P. glomerata* and *P. prunicola* as described by Wollenweber and Hochapfel in 1936.



FIGS. 1–6. Time sequence photomicrographs of dictyoporospore formation in *Alternaria tenuis*, QM 586. FIG. 1. 8:15 P.M. FIG. 2. 8:40 P.M. FIG. 3. 8:45 P.M. FIG. 4. 8:50 P.M. FIG. 5. 8:55 P.M. FIG. 6. 9:05 P.M. Light microscope used directly over colony surface. Approximate magnification, × 1164.

THE DICTYOSPORE

A dictyospore is a spore having both transverse and longitudinal septa. The spores of *Alternaria* and *Peyronellaea* are both included in this definition. This superficial grouping upon the basis of spore septation, while perhaps convenient, brings together two spore types of entirely different origin, union, and structure. It has resulted in the erection of such species as *Alternaria polymorpha* Planchon (**24**), *A. fumaginoides* Peyronel (**11**), and *A. hominis* Agostini and Tredici (**1**). These species have been erroneously erected upon the dictyospore stage of species belonging to the genus *Peyronellaea*. Bolle (**5**) has pointed



FIGS. 7–12.

FIGS. 7–9. Phase-contrast photomicrographs of conidia of *Alternaria tenuis*, QM 586. FIG. 7. Young porospore attached to its conidiophore (×1164). FIG. 8. Chain of dictyoporospores attached to conidiophore (×1164). FIG. 9. Union of porospores (×666).

out that these dictyospores are not true conidia as found in the genus *Alternaria* but are chlamydosporic elements.

Mason (**22**) has termed the dictyospores of an organism that Wiltshire had called *Coniothecium scabrum* McAlp., dictyothallospores. This organism produces pycnidia and is undoubtedly, from the picture and description of Mason, a member of the dictyochlamydospore group (*Peyronellaea*). Mason's term dictyothallospore for these chlamydospores lacks the preciseness which the term dictyochlamydospore imparts.[2] It is not clear that Mason made a distinction between the two types of dictyospores under discussion at present.

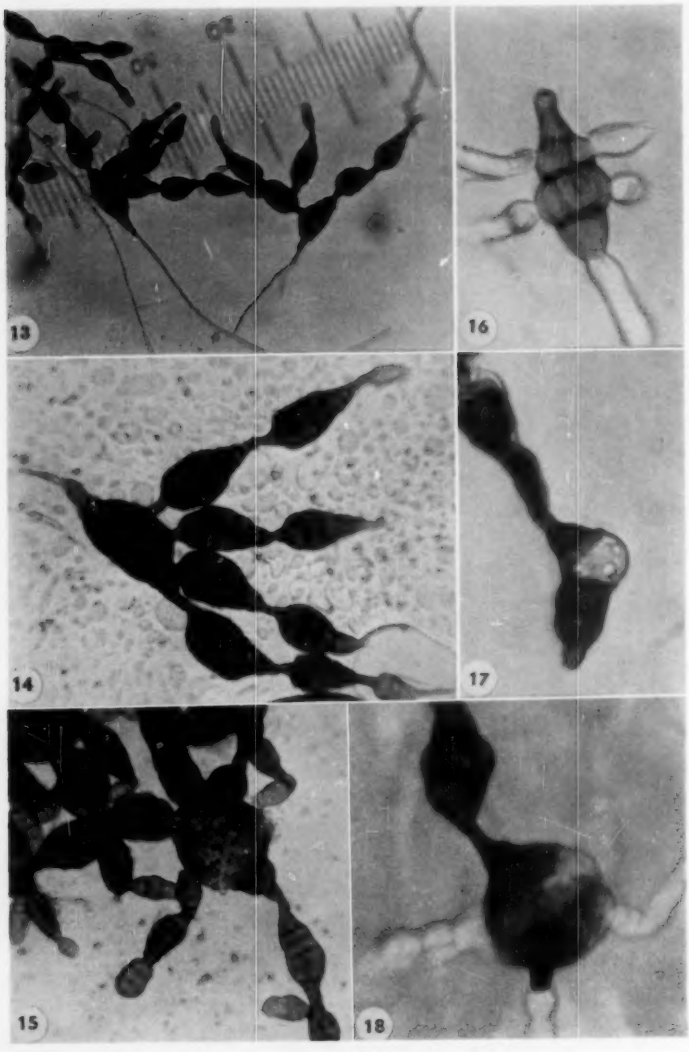
Langeron and Vanbreuseghem (**20**) placed the dictyothallospore of Mason and the *Alternaria* dictyospore, without differentiation, in their third group *Dictyospores*. Loubière (**21**) has recently discussed the *Alternaria* conidium as a type of blastospore—a *blastospore-dictyospore*. Goos (**15**) has argued that ". . . the conidia of *Alternaria* are borne on distinct conidiophores, and are, therefore, conidiospores and should not be included as thallospores. In general, the term dictyospore fails to designate a spore distinct by its mode of development, and serves only as a useful descriptive term." If, as Goos infers, we need a term to designate the *Alternaria* spore by its mode of development, we have one by simply applying the prefix *dictyo* to Hughes's (**18**) term porospore of group VI. Thus we arrive at a spore term meaningful by its mode of origin, as is the term dictyochlamydospore in the case of *Peyronellaea* spores.

The dictyospores of *Alternaria* and *Peyronellaea* serve as excellent examples of spores formed by two different types of development. In *Alternaria* the dictyospore is an exogenous spore not originating through the transformation of the existing thallus. In *Peyronellaea* the dictyospore is an endogenous spore formed by the transformation of the existing thallus.

Ainsworth and Bisby (**2**) define a chlamydospore as "a thick-walled, non-deciduous, intercalary or terminal asexual spore made by the rounding up of a cell or cells . . . ," and a *conidium verum* as "any

[2] Goos (**15**) uses the term thallospore in a generic sense and chlamydospore in a more specific sense.

FIGS. 10–12. Dictyochlamydospore formation in *Peyronellaea glomerata*. FIG. 10. Swelling of terminal hypha preceding septation (× 1000). FIG. 11. Early stage in which cross walls have formed (light microscope, × 500). FIG. 12. Mature dictyochlamydospores. Compare the broad union between spores and the characteristic ragged rupture of the cell wall in the lower spore with that of the dictyoporospores of FIG. 9 (× 877).



FIGS. 13–18.

FIGS. 13–14. Chains of dictyochlamydospores which superficially resemble *Alternaria* conidia. Note, however, the broad union between spores and the difference in symmetry between the young terminal spores in FIG. 13 and those of FIGS. 1–9. FIG. 13, × 500. FIG. 14, × 750.

asexual spore (but not a sporangiospore or intercalary chlamydospore) . . . which when mature comes away from its conidiophore. . . ." In these definitions a *conidium verum* is essentially deciduous and a chlamydospore non-deciduous. Upon the basis of these definitions the deciduous *Alternaria* conidium may be separated from the non-deciduous dictyochlamydospore of *Peyronellaea*.

Hughes (**18**) has drawn attention to what may be the key to a realistic approach to classification in the Fungi Imperfecti. He has established a number of sections of the imperfects upon his belief that ". . . there are only a limited number of methods whereby conidia can develop from other cells and that morphologically related imperfect states will only be brought together when the precise methods of conidium origin take first place in the delimitation of the major groupings." This concept of the importance of conidium origin may be utilized to differentiate the dictyospore of *Alternaria* from the dictyospore of *Peyronellaea*.

In *Alternaria* the origin of a new spore appears to be through a small pore dissolved in the beak of the preceding spore (**27**).[3] Through this pore the protoplasm flows enclosed in a thin membrane. This spherical vesicle rapidly becomes elongated and a flexible new cell wall gradually develops (FIGS. 1–6). The delicate link between successive spores is through this membrane. In a mature *Alternaria* spore the only sign left of attachment to the preceding spore is a minute scar at the spore base. This type of spore has been referred to by Hughes as a *porospore*.

In the genus *Peyronellaea* the dictyochlamydospore originates endogenously by the elongation and broadening of the blunt growing tip of a terminal hypha or preceding spore (FIGS. 10, 11, and 13).[4] The broad union between dictyochlamydospores when broken appears as a

[3] The author is indebted to Dr. Emory G. Simmons of the U. S. Quartermaster Research and Development Command, Natick, Massachusetts, for initially pointing out the differences existing between the origin of the dictyospore of *Alternaria* and the dictyospore of *Peyronellaea*.

[4] Chippendale (**7**) and Mercer (**23**) have made similar observations.

FIG. 15. A "micropycnidium" in a chain of dictyochlamydospores. This structure results from the alteration of a dictyochlamydospore in the aerial spore chain. These micropycnidia produce typical pycnidiospores (× 750).

FIG. 16. Germinating dictyoporospore (× 1000).

FIG. 17. Germinating dictyochlamydospore emerging from ruptured outer spore wall (× 1000).

FIG. 18. Germinating dictyochlamydospore transforming into a pycnidium twelve hours after inoculation onto agar.

ragged rupture of the connecting cell wall and contrasts markedly with the smooth break which occurs between porospores (FIGS. 9 and 12).

The endogenous component cells of a mature dictyochlamydospore are loosely associated and may be separated from each other and from the outer wall of the dictyochlamydospore by gently crushing mature spores on a slide under a cover slip. The elements of an *Alternaria* spore are firmly united and can not be mechanically separated. When the dictyochlamydospore germinates the component cells swell and rupture the outer wall (FIG. 17). In the *Alternaria* spore individual elements of the spore appear to germinate by dissolving a pore in the cell wall (FIG. 16). In a number of germinating dictyochlamydospores, pycnidia arise directly from the compound cellular mass (FIG. 18). *Alternaria* spores upon germination never give rise to pycnidia. Miniature pycnidia may be found between elements in a chain of dictyochlamydospores (FIG. 15). Pycnidia are not known to occur in pure cultures of any organism that produces true *Alternaria* porospores (**5**).

SUMMARY

The differences which exist between the dictyochlamydospore of *Peyronellaea* and the dictyoporospore of *Alternaria* are summarized as follows:

1. The dictyochlamydospore of *Peyronellaea* is a non-deciduous spore fitting the general definition of a thallospore and more specifically that of a chlamydospore. When the elements in a spore chain break apart a ragged edge is left on each spore indicating the place of attachment to the previous spore.

2. The dictyochlamydospore is composed of an outer wall which is separable from the walls of the component cells within the compound spore. The component cells of the dictyochlamydospore are rather easily separable from each other. Miniature pycnidia may occur between the elements in a dictyochlamydospore chain.

3. The *Alternaria* spore is a deciduous spore and fits the definition of a conidium and porospore. When the elements in a spore chain break apart the only indication of attachment to the previous spore is by an almost imperceptible scar at the base and the beak of the spore.

4. The *Alternaria* spore does not appear to be separable into an outer and an inner spore wall and the component cells of the dictyoporospore remain firmly united to one another. Pycnidia are not known to occur in the life cycle of any true *Alternaria* species.

The genus *Peyronellaea* has been erected upon the presence of two

spore forms present in the life cycle of its members. The pycnidial stage is ascribable to the genus *Phoma*, where it lies among many nondescript organisms of similar fructification. The chlamydosporic stage is distinct and easily recognizable.

The term dictyospore is a general term lacking precision in respect to describing mode of spore origin and has been used erroneously to group together entirely different spore types. The dictyospores of *Alternaria* and *Peyronellaea* have been used as examples in demonstrating the superficial resemblance of dictyoporospores and dictyochlamydospores when contrasted with their mode of origin, structural difference, and manner of germination.

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A NEW GENUS OF THE MONILIACEAE[1]

J. C. EDWARD[2]

(WITH 2 FIGURES)

An unknown fungus was isolated frequently during the warmer months of the year from farm soil of the Allahabad Agricultural Institute (Edward and Srivastava, **1957**), and is here described as a new genus and species.

The fungus grows equally well on Czapek's and potato-dextrose agar. Its optimum temperature and pH for growth are about 32° C and 4.5, respectively. The morphological characteristics of the fungus have, thus far, remained stable through frequent subculturing. The hyphae are septate, at first hyaline, later turning subhyaline to pale brown. Older colonies are floccose with a thick, matted basal felt, pale grayish-brown on the surface and black on the reverse. The conidiophores develop in 4- to 5-day-old cultures. They arise from a basal hyphal cell, which is relatively thick-walled with dark, smooth to warted surface (FIG. 1C). The conidiophore is unbranched, erect, septate, and colored, with papillate outgrowths on the surface, more pronounced toward the basal region (FIG. 1A). It is narrower and lighter in color toward the apex and invariably ends in a phialide. The phialides are simple, sparsely echinulose, flask-shaped with a broad base, hyaline to subhyaline, and are normally borne in whorls at or near the top of the conidiophore (FIGS. 1B, 2). The free end of the phialide is attenuated and produces a long chain of conidia in basipetal succession (FIG. 1B). Sessile phialides occurring on the creeping hyphae are more common in very young cultures (FIG. 1D). Rarely, they may be partly divided, giving rise to 2, or less commonly 3, phialides (FIG. 1E). The hyaline to subhyaline conidia are produced in long chains, one-celled, finely echinulose, broadly ellipsoid, with fine, small, connectives at each end (FIG. 1F). The conidia germinate within 4 hours in distilled water at

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room temperature. They produce 1 or 2 germ tubes which arise from the pointed ends or from the central region (FIG. 1G).

Reference to relevant literature has shown that this fungus is closely related to the genus *Paecilomyces* (Brown and Smith, **1957**; Hughes,

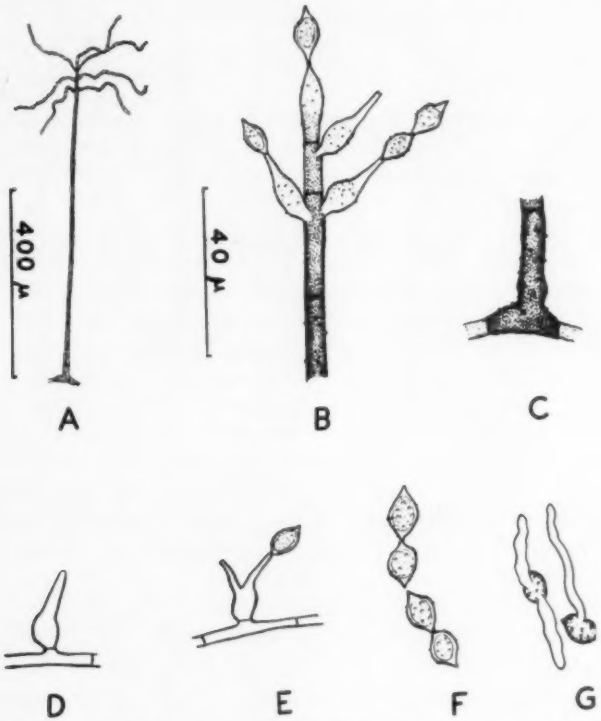

FIG. 1. *Acrophialophora nainiana*. A. Conidiophore with phialides bearing catenate phialospores. B. Top portion of the conidiophore bearing verticils of flask-shaped phialides. C. Basal hyphal cell with lower part of the conidiophore. D. Sessile phialide. E. Sessile phialide divided. F. Broken chain of phialospores. G. Germinating phialospores.

1951). The characteristics of similarity are: the catenate conidia, their development from phialides, and the occurrence of sessile phialides on creeping hyphae in addition to those borne on the conidiophores. The sessile phialides produced by this fungus are, however, morphologically more similar to those of *Masonia* (Smith, **1952**). Despite these simi-

larities, there are important differences which make this fungus distinct from *Paecilomyces*. They are: the occurrence of a definite basal hyphal cell from which the conidiophore arises (FIG. 1C); the characteristic unbranched, erect, colored conidiophores which are fertile only toward

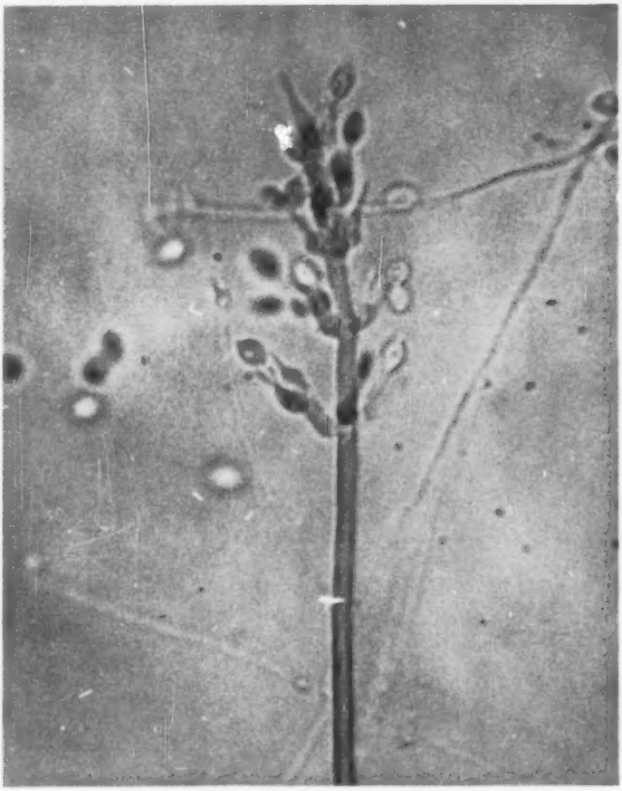

FIG. 2. *A. nainiana*. Top portion of the conidiophore-bearing phialides.

the top portion; the distinct swelling found at the base of the phialide; and the tips of the phialides, which do not curve or bend away from the main axis. Based on these facts the fungus is described as a species of a new genus. The generic name proposed for the fungus is *Acrophialophora* to indicate that the phialides are borne toward the top region of the conidiophore. As the fungus was isolated from the farm

soil of the Allahabad Agricultural Institute, situated at the locality, Naini, India, the name *Acrophialophora nainiana* is proposed for the species.

ACROPHIALOPHORA gen. nov.

Pertinet ad Fungos Imperfectos, Moniliales, Moniliaceas, Hyalosporas; conidiophoris erectis, coloratis, septatis, fertilibus ad summum, ubi paucis septis, non ramosis; phialidibus ampullaceis, simplicibus, in verticillis in conidiophoro, etiam in hyphis repentibus gestis; phialosporis hyalinis, continuis, catenariis, in successione ab apice ad basim genitis.

Fungus Imperfectus, Moniliales, Moniliaceae, Hyalosporae; conidiophores unbranched, erect, colored, septate, fertile toward the few-septate top; phialides flask-shaped, simple, in verticils on the conidiophore, also borne on creeping vegetative hyphae; phialospores hyaline, continuous, catenate, produced in basipetal succession.

Acrophialophora nainiana sp. nov.

Coloniis in agaro Czapekii et in agro 'potato-dextrose' ad 30° C crescentibus 30 mm in septem diebus, consistentibus in coactis densis concretis basilaribus cum hyphis floccosis aeriis et cum margine aliquantulo inaequali, superficie primo subalba, deinde pallide griseola-fulva, reversa nigra, sine odore; hyphis hyalinis in pallide fulvum desinentibus, septatis, 1.5–4.2 μ diam.; cella basilari conidiophorum gerente phaea, crasse tunicata, cum superficie levi vel verrucosa, 6.4–16.2 μ long. et 2.5–3.5 μ diam.; conidiophoris non ramosis, erectis, 20–40-septatis, fertilibus ad 4–10-septatum summum, fuscis ad basim, expalentibus in apice, muro crasso cum papillis manifestis brevibus ad basim, ad 4.5 μ diam. basi, attenuentibus ad 1.5–3.0 μ in apice, desinentibus semper in phialide 500–1500 μ long.; phialidibus in conidiophora, hyalinis vel subhyalinis cum superficie sparse echinulata, in verticillis ad quattuor raro pluris, ampullaceis cum basi in medio afflata, 4.5–9.0 × 3.0–9.5 μ, ulteriore altera vel tertia parte longitudinis in tubulo angusto fastigante 0.5–1.0 μ diam., extremis rectis raro divisis, etiam in hyphis repentibus; phialosporis late ellipsoideis, hyalinis vel subhyalinis, utroque apiculatis, subechinulatis, 3.0–9.0 × 1.5–4.5 μ, in catenis implicatis 200–300 μ long.

Colonies on Czapek's and potato-dextrose agar at 30° C growing 30 mm in 7 days, consisting of a dense, matted basal felt with floccose aerial hyphae and a somewhat irregular margin, surface at first dull white, later turning pale grayish-brown, reverse black, odor nil; hyphae hyaline to pale brown, septate, 1.5–4.2 μ in diam.; a basal hyphal cell from which a conidiophore arises present, dark-colored, thick-walled with smooth to warted surface, 6.4–16.2 μ long and 2.5–3.5 μ wide; conidiophores unbranched, erect, 20–40-septate, fertile at the top 4–10 septations, dark brown toward the base becoming lighter at the apex, wall thick with pronounced short papillary outgrowths toward the base, up to 4.5 μ in diam. at the base, diminishing to 1.5–3.0 μ at the apex, invariably ending in a phialide, 500–1500 μ long; phialides on the conidio-

phore, hyaline to subhyaline with a finely echinulated surface, in verticils of up to 4 or rarely more, flask-shaped with the base swollen in the middle, 4.5–9.0 × 3.0–9.5 μ, the last one-half to one-third of the length tapering to a narrow tube 0.5–1.0 μ in diam., distal ends straight, rarely divided, also occurring on creeping hyphae; phialospores broadly ellipsoid, hyaline to subhyaline, with a fine connective at each end, faintly echinulate, 3.0–9.0 × 1.5–4.5 μ in chains 200–300 μ long which become entwined.

The TYPE is represented by a single-spore culture of the fungus isolated from the farm soil of the Allahabad Agricultural Institute, India. The type culture has been deposited in the Commonwealth Mycological Institute, Kew, Surrey, England; the Department of Plant Pathology and Botany, University of Minnesota, St. Paul, Minnesota, U.S.A.; the Department of Plant Pathology, Bacteriology, and Entomology, West Virginia University, Morgantown, West Virginia, U.S.A.; and the Department of Biology, Allahabad Agricultural Institute, Allahabad, India.

ACKNOWLEDGMENTS

I wish to express my thanks to Dr. H. L. Barnett, West Virginia University, Dr. J. C. Gilman, Iowa State College, Dr. S. J. Hughes, Canada Department of Agriculture, Ottawa, and Dr. G. Smith, London School of Hygiene and Tropical Medicine, for examining the fungus and giving useful suggestions; Dr. J. J. Christensen, University of Minnesota, for his encouragement in the investigation, Dr. R. D. Durbin, University of Minnesota, for his valuable suggestions and help in the preparation of this paper, Mr. T. Kavanagh, University of Minnesota, for rendering the Latin diagnosis, and Mr. T. D. Wyllie, University of Minnesota, for the photograph.

SUMMARY

An unknown moniliaceous fungus isolated from soil is described and assigned to a new genus, *Acrophialophora*. The genus is distinguished from *Paecilomyces* in producing unbranched, colored, erect conidiophores which are fertile only toward the top region. The species is designated *Acrophialophora nainiana*.

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SEVERAL ZOOPAGACEAE SUBSISTING ON A NEMATODE AND ON SOME TERRICOLOUS AMOEBAE

CHARLES DRECHSLER[1]

(WITH 10 FIGURES)

The several fungi herein described as new members of the Zoopagaceae were obtained from softened or discolored roots and from plant detritus slowly decomposing on the ground. Though all are thus of terrestrial origin, one of them is assigned to a genus that was erected rather casually, without a diagnosis or definition, on a species that developed under aquatic conditions. As the species, moreover, was held by its author to belong most probably in the Mucedinaceae the genus has not hitherto been subsumed under the Zoopagales or, indeed, under any other order of the Phycomycetes. In addition to the descriptions of the several new fungi, supplementary information on a few known species of the Zoopagaceae is supplied.

Euryancale marsipospora sp. nov. (μάρσῐπος, pouch; σπορά, seed)

Mycelio assumenti in vermiculo nematoideo crescente, incolorato, in hyphis continuis plerumque 3–4.5 μ latis consistente; hyphis genitabilibus ex animali mortuo diverse porrectis, continuis, incoloratis, repentibus, plerumque 1–1.8 μ latis, ramulos fertiles proferentibus; ramulis fertilibus inter se vulgo 15–40 μ distantibus, 20–45 μ longis, basi repentibus et 1–1.2 μ latis, medio in aerem abrupte recurventibus et 2–2.5 μ latis, apice circa 1.2 μ latis et ibi conidia deinceps gerentibus; conidiis incoloratis, in cellula viventi et sacculo inani constantibus,—cellula viventi arcuata, 7–9 μ longa, 1.2–1.6 μ lata,—sacculo inani vulgo 2–2.5 μ longo, 1.5–1.8 μ lato.

Vermiculos nematoideos (*Rhabditis* sp.) interficiens habitat in foliis quercorum putrescentibus prope College Park, Maryland, etiam in foliis arborum (*Aceris, Betulae, Ulmi*) putrescentibus prope Butternut, Wisconsin. Typus: Figura 1, A. E.

Mycelium developing in living nematodes and soon killing the animals, usually somewhat branched, colorless, composed of assimilative hyphae 3 to 4.5 μ wide; reproductive hyphae extended in different directions from the dead host nematode, continuous, colorless, procumbent, 1 to 1.8 μ (mostly about 1.2 μ) wide, bearing conidiiferous branches

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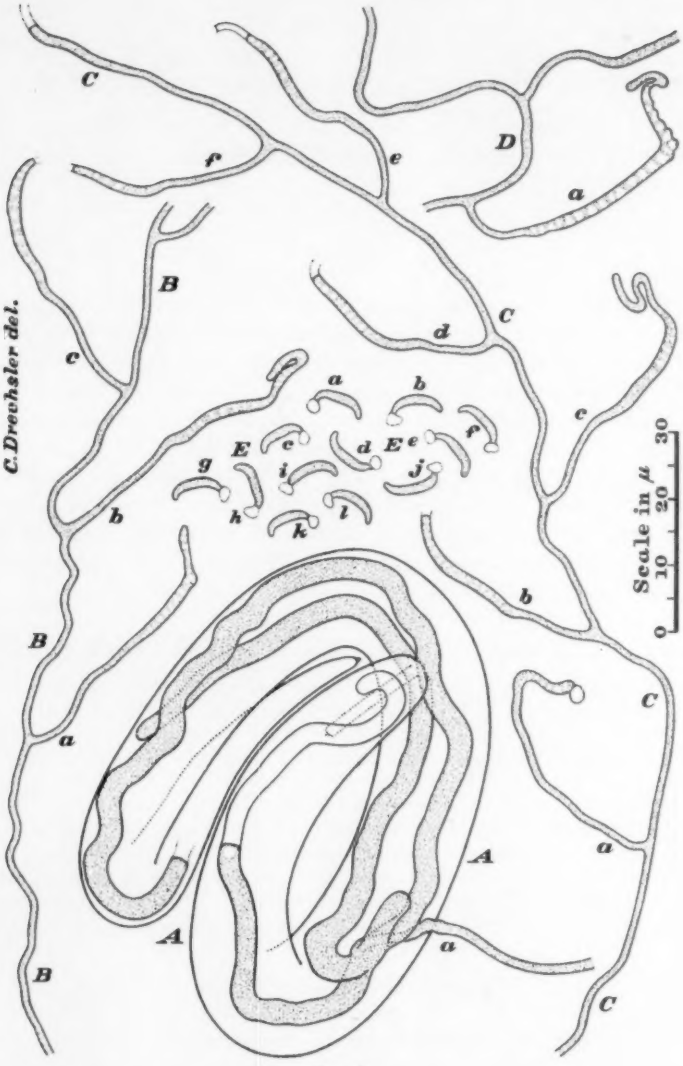

FIG. 1.

FIG. 1. *Euryancale marsipospora*, drawn at a uniform magnification with the aid of a camera lucida, from a preparation covered with a cover glass; ×1000. *A*, Infected eelworm (*Rhabditis* sp.) showing internal assimilative hyphae and

commonly at intervals of 15 to 40 μ; conidiiferous branches mostly 20 to 45 μ in total length, consisting of a procumbent proximal portion 1 to 1.2 μ wide, together with an expanded median portion 2 to 2.5 μ wide, which curves upward into the air and tapers to a width of about 1.2 μ at the tip, where conidia are produced one after another; conidia colorless, consisting of an arcuate living cell usually 7 to 9 μ long and 1.2 to 1.6 μ wide, and bearing at one end an empty membranous pouch commonly 2 to 2.5 μ long and 1.5 to 1.8 μ wide.

Euryancale marsipospora was found developing rather sparingly in a maize-meal-agar plate culture which after being permeated by mycelium of my *Pythium sclerotelchum* had been further planted with a small quantity of leaf mold collected in an oak (*Quercus* spp.) wood near College Park on March 18, 1958. Only 16 individual eelworms were observed undergoing destruction by the fungus, and it seems probable that the total number of animals killed, including some that could well have been concealed under a deposit of the opaque forest detritus, may not have exceeded 25. The infected animals succumbed in variously coiled postures at different depths below the surface of the agar. Two specimens which were only shallowly submerged and still showed much of their original structure, were kindly identified by A. L. Taylor as belonging to a species of *Rhabditis*.

The sturdy hyphae that grow lengthwise through an infected eelworm become rather clearly visible after they have largely assimilated the fleshy tissues permeated by them. Usually the smaller animals (FIG. 1, *A*) contain only 1 or 2 trophic hyphae in any region, whereas 5 to 7 such hyphae may be present in the wider portions of animals measuring more than 500 μ in length. Hyphal branching, as might be expected, is more freely displayed in larger animals than in small ones. In giving rise to one (FIG. 1, *A*, *a*) or more reproductive hyphae the assimilative mycelium becomes progressively emptied of protoplasmic materials, and at intervals cross-walls are laid down to mark successive stages of evacuation. The tubular membranes disappear soon after being emptied, so that eventually all of the mycelium vanishes from sight, as does also the equally empty host integument.

proximal portion of a reproductive hypha, *a*. *B*, Portion of reproductive hypha with 3 conidiiferous branches, *a–c*, of which the distal portions have incurred some distortion, evanescence and degeneration. *C*, Portion of reproductive hypha with 6 conidiiferous branches, *a–f*, among which one, *c*, shows the recurved aerial termination, while in others the aerial termination has evanesced. *D*, Portion of reproductive hypha bearing a conidiiferous branch, *a*, with 6 protuberances marking positions where 6 conidia had been borne successively. *E*, Conidia, *a–l*, showing usual variations in size and shape.

When undisturbed asexual reproductive apparatus of *Euryancale marsipospora* is viewed under a microscope with a dry objective, the upcurved aerial tips of the conidiiferous branches form a delicate lacelike pattern much like that presented by sporulating material of *E. sacciospora* Drechsler (**1939**). Unfortunately, when a cover glass is applied to facilitate scrutiny under an immersion objective of higher magnification, the conidiiferous branches (FIG. 1, *B, a–c; C, a–f; D, a*) not only become distorted, but also in most instances incur rapid degeneration and evanescence of the distal portion most directly concerned in spore formation. Consequently, with only meager material available, it was not possible to establish certainly that the empty membranous pouch visible on each conidium (FIG. 1, *E, a–l*) is attached at the basal end rather than at the tip. In some few conidiiferous branches in which the distal portion appeared less seriously damaged (FIG. 1, *C, c; D, a*) than in most others the arrangement of parts suggested that the empty appendage here develops, much as in *E. sacciospora*, at the proximal end of the spore. At all events the detached conidia are unusually distinctive despite their simple design and small dimensions. They are fully adequate for identification of the parasite once their connection with continuous assimilative hyphae in a nematode has been ascertained. As such connection was earlier revealed by a fungus with entirely similar conidia (Drechsler, **1951**: p. 182, fig. 6, *H;* p. 184–185) that developed sparingly on maize-meal agar planted with leaf mold from deciduous woods in northern Wisconsin, the locality where the leaf mold was collected merits citation in the statement on the known distribution of *E. marsipospora*.

Amoebophilus sicyosporus sp. nov. (*σίκυος*, cucumber; *σπόρος*, seed)

Ectoparasiticus, sine hyphis sterilibus filiformibus. Conidiis incoloratis, aliquantulum asperis, cucumiformibus (fructui *Cucumeris sativi* similibus), basi vulgo minute acuminatis sed apice vel rotundatis vel minute acuminatis, plerumque 4–24 μ longis, 1.8–2.7 μ latis, uno extremo ad *Amoebam* inhaerentibus, ibi haustorium pediculatum in animal intrudentibus, mox prope extremum affixum et ex altero extremo nova conidia singulatim vel in catenula gignentibus, postea animali debilitato hyphas zygosporiferas emittentibus; haustoriis globosis vel bilobosis vel trilobosis, 1–2.5 μ longis, 1–4 μ latis, pediculo plerumque 0.6–1.5 μ longo et 0.4–0.5 μ lato praeditis; hyphis zygosporiferis incoloratis, filiformibus, nexuosis, saepius 5–22 μ longis, 0.9–1.5 μ latis; zygosporis vulgo 3–20 prope animal emortuum oriundis, primo globosis, 5–8 μ in diametro, mox in maturitate multangulis, flavidis, cellulam viventem 3.7–5.5 μ crassam circumdantibus, postrema in apice singulati tubuli germinationis 2–3.5 μ longi et 0.5–0.8 μ crassi unum conidium vel 2–3 conidia catenata gignentibus.

Amoebam vulgo 20–40 μ latam (*Amoebae guttulae* similem) interficiens habitat in radicibus putrescentibus *Polygoni pensylvanici* et *Glycinis macis* prope Beltsville, Maryland. Typus: Figura 2.

Ectoparasitic, devoid of filamentous mycelial hyphae. Conidia colorless, very slightly rough, of cylindrical shape somewhat like that of cucumber (*Cucumis sativus* L.) fruits, often minutely pointed at the base, rounded or minutely pointed at the tip, mostly 4 to 24 μ long, 1.8 to 2.7 μ wide, adhering by one end to an amoeba and from this end intruding a pedicellate haustorium into the animal, soon afterwards from positions near the affixed end and at the free end giving rise to new conidia singly or in chains, subsequently (on disablement of the animal) putting forth zygophoric hyphae; haustoria globose or bilobate or trilobate, mostly 1 to 2.5 μ long, 1 to 4 μ wide, supported on a pedicel commonly 0.6 to 1.5 μ long and 0.4 to 0.5 μ wide; zygophoric hyphae colorless, filamentous, more or less intertangled, 5 to 22 μ long, 0.9 to 1.5 μ wide, uniting in pairs and at the junction producing a zygospore; zygospores formed in a group of 3 to 20 near the parasitized animal, first globose, 5 to 8 μ in diameter, then at maturity pronouncedly polyhedral, yellowish, loosely enclosing a spherical protoplast 3.7 to 5.5 μ in diameter, ultimately germinating by producing a single conidium or 2 or 3 catenated conidia on a tubular pedicel usually 2 to 3.5 μ long and 0.5 to 0.8 μ wide.

Amoebophilus sicyosporus developed in 14 isolation plate cultures prepared by planting on maize-meal agar, without any use of surface disinfectants, discolored roots of pinkweed (*Polygonum pensylvanicum* L.) and soybean (*Glycine max* (L.) Merr.) collected near Beltsville on August 22, 1958. The cultures were protected against evaporation by being stored under an inverted battery jar. Although the mixtures of microorganisms that became established in the several Petri dishes differed noticeably, the agar substratum underwent gradual softening in all cultures alike, until after 20 days it was everywhere of semi-fluid consistency. The somewhat aquatic conditions thus brought about are believed to have been of primary importance in encouraging development of *A. sicyosporus*. When the cultures were 50 days old, some revealed the fungus active as a parasite and concomitantly giving rise to conidia and zygospores, while others displayed it only in groups of resting or germinating zygospores.

Active individuals of the amoeba habitually attacked by *Amoebophilus sicyosporus* are usually elongated in the direction of their movement (FIG. 2, *A, B*). The largest specimens may then measure 60 μ in length and 20 μ in average width (FIG. 2, *A*). Relatively inactive individuals that have assumed a somewhat rounded shape (FIG. 2, *C–H*) commonly vary from 15 to 30 μ in length and width. Each animal

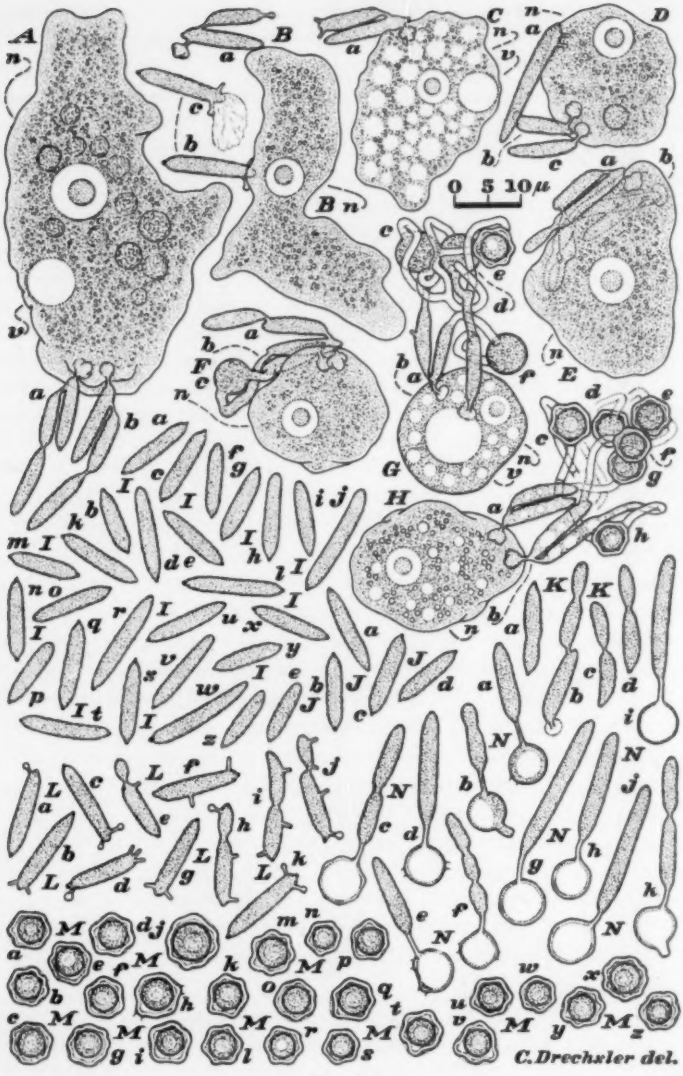

FIG. 2.

FIG. 2. *Amoebophilus sicyosporus*, drawn at a uniform magnification with the aid of a camera lucida; × 1000. *A*, Active host amoeba parasitized by 2 implanted conidia, *a–b*, each connected near its affixed end with a chain of 2 undelimited

shows a single globose nucleus (FIG. 2, *A–H: n*) measuring 5 to 9 μ in diameter and containing a slightly darker endosome mostly 2.5 to 4 μ in diameter. A contractile vacuole (FIG. 2, *A, v; C, v; G, v*) can be distinguished in some animals. In respect to dimensions and nuclear structure the amoeba corresponds approximately with the description of *Amoeba guttula* Dujardin given by Penard (**1902**).

Amoebophilus sicyosporus, like *Bdellospora helicoides* Drechsler (**1935a**), may properly be considered an ectoparasite since it forms no massive vegetative body within the host but merely intrudes a small haustorium from which all assimilated materials are promptly removed to be used for external development. While the haustorium of *B. helicoides* is of a design found in many predacious Zoopagaceae, that of *Amoebophilus sicyosporus* is of an unusual type hitherto observed, among fungi described as members of the Zoopagaceae, only in *Zoopage phanera* Drechsler (**1935a**). Unmistakably, however, the same lobate type of haustorium (gelappte Blase) was described and figured by Geitler (**1937**) in his account of a parasitic fungus he found occurring as filamentous appendages on *Amoeba proteus* Leidy taken from slimy mud near the Lunz Biological Station in Austria. Geitler recognized a

conidia; *n*, nucleus of host; *v*, contractile vacuole. *B*, Active host amoeba to which is shown adhering an uprooted conidium, *a*, with an undelimited daughter conidium in parabasal position; *b*, a conidium still adhering but about to be disengaged from animal; *c*, same conidium after its disengagement 10 minutes later, showing a fragment of pellicle affixed to it; *n*, host nucleus. *C*, Quiescent host amoeba with an implanted conidium, *a*, to which an undelimited daughter conidium is attached near the base; *n*, host nucleus; *v*, contractile vacuole. *D*, Inactive host animal showing one conidium, *a*, securing a foothold, and 2 implanted conidia, *b–c*; *n*, host nucleus. *E*, Inactive host animal parasitized by 2 implanted conidia, *a–b*, with which 4 and 3 daughter conidia, respectively, are connected; *n*, host nucleus. *F*, Quiescent host animal parasitized by 2 implanted conidia, *a–b*, which together have put forth a pair of sexual hyphae and produced a zygospore, *c*; on *a*, besides, is borne a chain of 2 undelimited conidia; *n*, host nucleus. *G*, Inactive host amoeba parasitized by 2 implanted conidia, *a–b*, which together with a daughter conidium produced by *a* have extended sexual hyphae and given rise to 4 zygospores, *c–f*; *n*, host nucleus; *v*, enlarged vacuole. *H*, Inactive host amoeba parasitized by 2 implanted conidia, *a–b*, each connected with a daughter conidium—all 4 conidia having extended sexual hyphae and together produced 6 zygospores, *c–h*; *n*, host nucleus. *I* (*a–z*), *J* (*a–e*), Detached cucumiform conidia showing usual variations in shape and size. *K*, Detached lobate conidium, *a*; *b*, uprooted conidium bearing distally a lobate conidium; *c–d*, lobate or interjoined conidia. *L*, Detached conidia, *a–k*, bearing one or more incipient haustoria betokening failure to become firmly implanted on a host animal. *M*, Mature zygospores, *a–z*, showing usual variations in size and shape. *N*, Zygospores in course of germination, *a–b*, and after germination, *c–k*.

like appressorium also in a small colorless body which Penard had earlier observed in specimens of his *Amoeba nobilis* parasitized by affixed fungus filaments. The colorless body, measuring scarcely 7 to 8 μ in diameter and bearing 2 or 3 distally lobed branches in stellate or trefoil arrangement, was reported by Penard to be immersed in the host protoplasm near a small tubular or horn-shaped, basal part from which the individual parasitic filament arose. Penard's view that the lobate body was a germinating spore free of the neighboring hyphal base had its explanation, according to Geitler, in failure to recognize a connection that was really present. Dangeard (**1910**) saw neither a haustorium nor an adhesive disc at the place where the "caudal appendages" he named *Amoebophilus Penardi* were affixed to the surface of his *Pelomyxa vorax*, but admitted that the manner of attachment needed further study. Fully three decades earlier Leidy (**1879**) had expressed similar perplexity with respect to the fixation of the "caudal filaments" of his *Ouramoeba vorax*. In a detached tuft the "root" appeared to Leidy to be continuous with a ball of homogeneous protoplasm. Although obvious resemblance led him to suspect that the "caudal filaments" might be fungus threads, he saw an important objection to such an interpretation in the fact that the filaments do not grow from a mycelium within the animal's body. This objection presumably had equal application to the more distinctly moniliform appendages which Leidy found attached to his *Ouramoeba botulicauda* and which in some dimensions differ rather little from conidial chains of *Amoebophilus sicyosporus*.

While a conidium of *Bdellospora helicoides* usually extends its haustorium into an amoeba host from a median position, a conidium of *Amoebophilus sicyosporus* more commonly becomes implanted by putting forth a haustorium somewhat obliquely from a position near one of its ends (FIG. 2, *A, a, b; C, a; D, a–c; E, a, b*). In *B. helicoides* the affixed conidium utilizes the materials obtained from the animal by swelling to greater size, whereas in *Amoebophilus sicyosporus* it employs all assimilated substance in reproduction immediately. During the earlier stages of infection when the protozoan host is still capable of active locomotion, asexual reproduction predominates strongly in *Amoebophilus sicyosporus*. The conidium newly anchored to the host usually puts forth 2 outgrowths about 0.4 μ wide, one being extended from a parabasal position and the other from an apical position. Each outgrowth, after attaining a length of 1 μ, widens rather abruptly and at the increased width elongates until an undelimited daughter conidium has been produced (FIG. 2, *A, a, b*). The daughter conidia may in turn give rise from an apical position, likewise without proximal delimitation, to a

conidium of the third generation, with the result that some chains of 3 undelimited spores are formed (FIG. 2, *E, a, b*). In my cultures such chains were less numerous than chains of 2 undelimited spores. Rather often an implanted conidium was found connected with no progeny other than a daughter of parabasal origin (FIG. 2, *C, a*), though the debilitated appearance of the host animal indicated prolonged parasitic attack and equally prolonged formation of new conidia. The large numbers of conidia strewn around haphazardly on the substratum (FIG. 2, *I, a–z; J, a–e; K, a–d; L, a–k*) provided good evidence that disjuction took place while the host animals were continuing their locomotion.

Very probably the strains and wrenches to which newly formed conidia of *Amoebophilus sicyosporus* are constantly subjected as the host animal keeps moving mainly bring about disjunction. Although now and then affixed conidia of the fungus are seen trailing at the posterior end of the host amoeba (FIG. 2, *A, a, b*) after the manner usual with the "caudal appendages" of aquatic rhizopods, more often they are found jammed against the side of the animal, protruding from its front, or pressed underneath it. Sometimes enough violence is exerted even to dislodge an implanted conidium provided with a well-developed haustorium and some apical (FIG. 2, *K, b*) or parabasal progeny (FIG. 2, *B, a*). When an adhering conidium (FIG. 2, *B, b*) becomes dislodged (FIG. 2, *B, c*), it may tear off and retain a small portion of the animal's pellicle, just as conidia of *Endocochlus binarius* Drechsler (**1949**), in being dislodged from *Amoeba papyracea* Penard, may carry away fragments of host pellicle.

Disjunction of a conidium in *Amoebophilus sicyosporus* is not known to be preceded by deposition of a basal delimiting wall. Ordinarily no partition is visible in the slender connections between an implanted conidium and all its attached asexual progeny of the first and second and third generations. Some detached conidia that are continuous distally with a young undersized spore (FIG. 2, *L, e, j*) must almost certainly have become abjointed when they were steadily receiving materials at the proximal end and conveying them to a growing young conidium at the tip. In the implanted conidium and its attached asexual progeny, somewhat as in actively elongating spore chains of *Alternaria*, continuous acropetal development of new conidia would seem combined with continued readiness for instant disjunction at nearly any or all isthmuses. That in such disjunction no injury is incurred either by the liberated conidium or by the conidium from which it was abjointed is due presumably to the minuteness of the disrupted passageway. An isthmus 0.4 μ in diameter between its outer contours would seem here usually to

surround a channel about 0.25 μ wide—a channel therefore only one-fourth to one-half as wide as the septal pores figured by Buller (**1933**, pp. 75–167) in illustrations of *Pyronema confluens* Tul. and found by him capable of being closed effectively against any encroachment of injury. Isthmuses wider than 0.5 or 0.6 μ, which if ruptured would leave exposed a broader channel, usually prove too sturdy to serve in disjunction. After fracture of a suitably slender isthmus its separated moieties occur as solidly filled stumps often too minute to attract attention. Accordingly when the abjointed conidium has been left behind, the reproductive apparatus implanted on the actively moving host animal has at the recently denuded tip only a relatively inconspicuous modification from which earlier development of a spore could be inferred.

The course of asexual reproduction in *Amoebophilus sicyosporus*, nevertheless, is fairly obvious when development proceeds in a soft agar culture, where all detached conidia remain in the positions where they were abjointed, constantly displaying their close resemblance to implanted conidia and the concomitant undelimited asexual progeny. In an aquatic culture, however, where detached conidia can float away and be lost to view as soon as they become abjointed, clues necessary for a correct interpretation might often be lacking, so that the observed parts of *Amoebophilus sicyosporus* or of any closely similar fungus might readily be misconstrued. The somewhat tufted moniliform filaments which Geitler found parasitizing *Amoeba proteus* and which, despite some similarities to the Leptomitaceae, he held most probably referable to the Cladochytridiaceae, appear to consist, beyond all reasonable doubt, of implanted conidia and their undelimited conidial progeny. The Austrian fungus seems therefore a species congeneric with *Amoebophilus sicyosporus*, but of more robust dimensions, its conidia evidently measuring 25 to 55 μ in length and 3 to 4 μ in width. Geitler's figures show as many as 6 daughter conidia arising in parabasal positions from an implanted conidium, and some of these parabasal daughters are catenated distally with one or two other conidia, though none of the implanted conidia are shown similarly provided with apical progeny. In contrast, only apical progeny is shown in Dangeard's figures of *Amoebophilus Penardi*, a species that likewise appears more robust than mine, as its "articles," or conidia, vary from 2 to 3 μ in width and may attain a length of 50 μ. A congeneric species with conidial apparatus of both apical and parabasal development may tentatively be recognized in the tufts of "caudal appendages" that Leidy found attached to his *Ouramoeba botulicauda* and set forth as consisting of "series of elongated elliptical bodies" resembling "strings of sausages." The bodies in question are

figured as tapering toward the proximal and distal ends less abruptly than conidia of *Amoebophilus sicyosporus* and might be characterized as being spindle-shaped rather than cucumber-shaped.

While a few constrictions appear in some figures given by Leidy (**1879**, Pl. IX, Fig. 5, 6) to illustrate the "caudal appendages" of his *Ouramoeba vorax*, other relevant figures (Leidy, **1879**, Pl. IX, Fig. 1, 2, 3, 10, 11) show long filaments of uniform width that bear little intimate resemblance to *Amoebophilus sicyosporus*. Further, although the filamentous appendages that Korotneff (**1879–1880**) found attached to his *Longicauda amoebina* and those that Penard observed on his *Amoeba nobilis* were named *Amoebophilus korotneffi* and *Amoebophilus caudatus*, respectively, by Dangeard, they lack the pronounced constrictions associated with the manner of conidial development and disjunction in my fungus. According to Korotneff the terminal branches of the filaments discussed by him ultimately acquired moniliform constrictions and their contents became converted into series of bodies presenting a refringent rather than a granular appearance. Dangeard regarded the components of the moniliform branches as spores. If, as appears likely, this view is correct, spore formation here might take place after the manner usual among the coarser species of *Cochlonema* and *Zoopage;* so that relationship with the Zoopagaceae would seem no less possible than with the Mucedinaceae. However, nothing suggestive of relationship in the Zoopagaceae is shown in the figures given by Penard (**1902**, p. 94, Fig. 4, 5) to illustrate his account of some thick-walled tapering appendages he found on his *Amoeba vespertilio* and considered analogous to those he had observed on *Amoeba nobilis*.

Amoebophilus sicyosporus reproduces sexually on host animals that have ceased to move about actively (FIG. 2, *F–H*). Two hyphae given off by separate conidia implanted close together (FIG. 2, *F, a, b*) conjugate apically and at the place of union give rise to a globose swelling, the young zygospore (FIG. 2, *F, c*). Through ramification of the first sexual hyphae and through production of new hyphae opportunity is provided for further conjugations, with the result that a cluster of zygospores (FIG. 2, *G, c–f; H, c–h*) progressively comes into being. Because of the confused intertanglement and early evanescence of the sexual branches, the connections between the small zygospores and their parent conidia are in most instances only partly discernible, yet here and there the origin of a zygospore (FIG. 2, *G, f; H, h*) from 2 separate conidia (FIG. 2, *G, a, b; H, a, b*) is clearly revealed. Although zygospores of similarly small dimensions are often encountered in scattered positions on agar substrata occupied by one of the more minute species

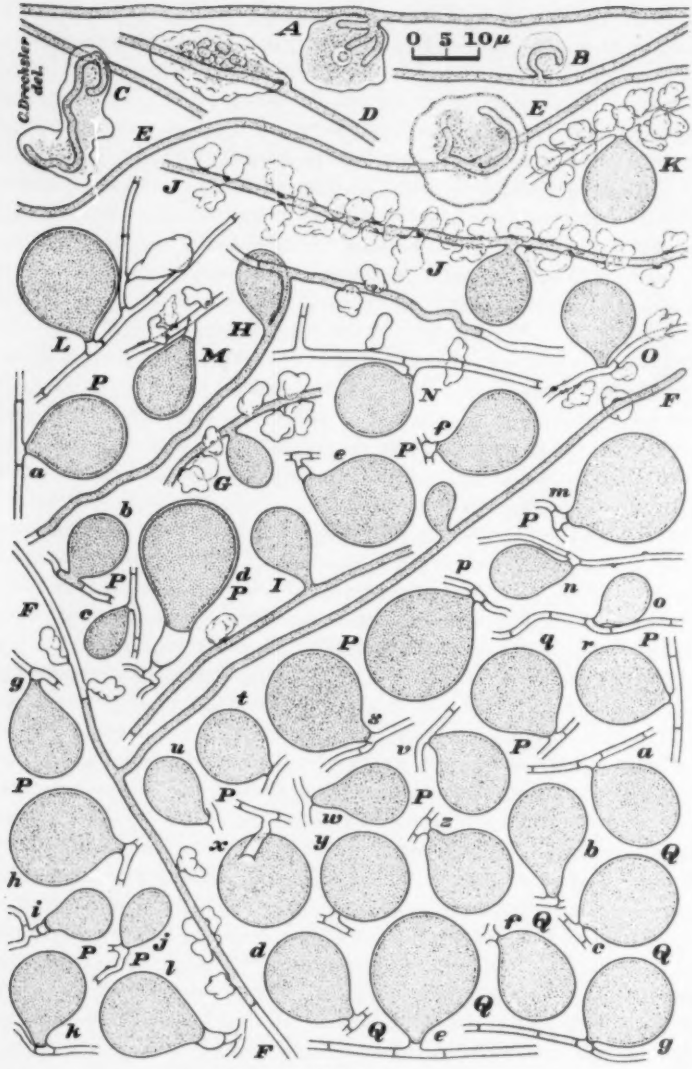

FIG. 3.

FIG. 3. *Cystopage sacciformis*, drawn at a uniform magnification with the aid of a camera lucida; × 1000. *A–C*, Portions of hyphae, each with a captured amoeba invaded by a haustorium. *D*, Portion of hypha with nearly depleted pellicle

of *Acaulopage*, the production of such small zygospores in clustered arrangement has been noted previously in the Zoopagaceae only in an unnamed endoparasitic form distinguished by a small U-shaped thallus (Drechsler, **1951**). With respect to structural make-up the mature zygospores of *Amoebophilus sicyosporus* (FIG. 2, *M*, *a–z*) show no marked departure from the stellate or polyhedral type most usual among members of the family. Their variations in size and shape are moderate. After a resting period of about 20 days many of them germinate spontaneously in the stale substratum where they were formed, without any addition of fresh water. Except possibly for somewhat greater dimensional variability the conidia resulting from such germination (FIG. 2, *N*, *a–k*) closely resemble those formed through asexual reproduction.

Cystopage sacciformis sp. nov.

Mycelio exiguo; hyphis ejus submersis vel procumbentibus, continuis, incoloratis, parce ramosis, plerumque 1–1.5 μ crassis, ad *Amoebas* inhaerentibus, animalia ita capientibus, pelliculam cujusque captivi perforantibus, haustorium intus evolventibus quod protoplasma exhaurit, a latere earum vel in apice rami plerumque 1–10 μ longi chlamydosporas ferentibus; haustorio in 1–3 ramis vulgo 5–20 μ longis et 1–1.2 μ latis constante; chlamydosporis incoloratis vel leviter flavidis vel leviter fumidis, quandoque globosis, semper apice late rotundatis, prope basim saepissime in pediculum rectum vel obliquum attenuatis, itaque vulgo utriformibus, plerumque 9–21 μ longis, 5–17 μ latis.

Amoebas vulgo 5–20 μ latas capiens consumensque habitat in radicibus *Polygoni pensylvanici* putrescentibus prope Beltsville, Maryland. Typus: Figura 3; Figura 4, A.

Mycelium scanty; its component hyphae submerged or prostrate, continuous, colorless, sparingly branched, mostly 1 to 1.5 μ wide, adhering to amoebae, thereby capturing the animals, then perforating the pellicle of each captive and intruding a haustorium that appropriates the fleshy contents, producing chlamydospores laterally or giving rise to such spores terminally on lateral branches 1 to 10 μ long; haustorium composed of 1 to 3 assimilative branches 5 to 20 μ long and 1 to 1.2 μ wide; chlamydospores colorless or slightly yellowish or slightly smoke-

of a captured amoeba. *E*, Portion of hypha from which 2 haustorial branches have been extended into a captured amoeba surrounded not by a membranous pellicle but by a thickish stiffened layer of ectoplasm. *F–J*, Simple and branched portions of living hyphae from which chlamydospores are arising and to which are affixed empty pellicles of captured amoebae in numbers of 6, 7, 2, 1 and 27, respectively. *K–O*, Empty portions of hyphae to which are attached a delimited chlamydospore and empty pellicles of captured amoebae in numbers of 13, 1, 3, 2 and 3, respectively. *P* (*a–z*), *Q* (*a–g*), Random assortment of chlamydospores, most of them in definitive state, showing attachment to parent hypha, as well as variations in size and shape.

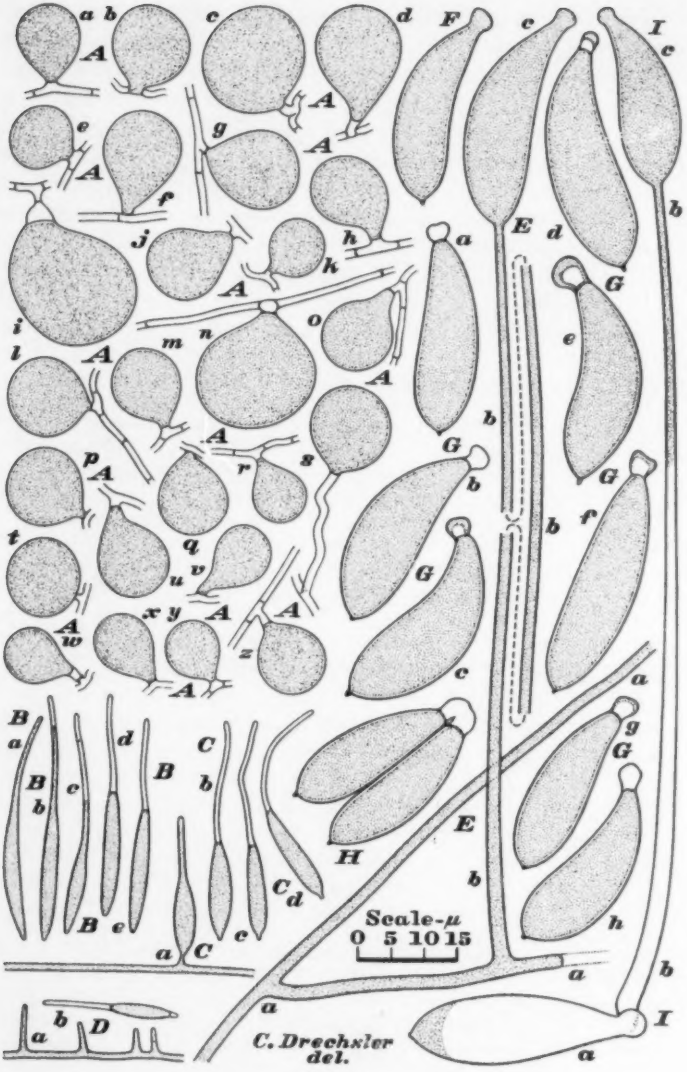

FIG. 4.

FIG. 4. Drawn at a uniform magnification with the aid of a camera lucida; × 1000. *A*, Chlamydospores, *a–z*, of *Cystopage sacciformis* from a soft maize-meal-agar plate culture 45 days old, showing attachment to parent hypha as well as

colored, always broadly rounded at the distal end, at the proximal end most often tapering into a straight or oblique neck, therefore commonly utriform, mostly 9 to 21 μ long and 5 to 17 μ wide.

Cystopage sacciformis appeared in several of the maize-meal-agar plate cultures which after being planted with discolored pinkweed roots collected on August 22, 1958, afforded development of *Amoebophilus sicyosporus*. When the cultures were 38 days old rather few living amoebae were found affixed to its hyphae (FIG. 3, *A*, *B*). In some of the larger captives (FIG. 3, *A*) a spherical nucleus measuring about 3 μ in diameter and containing an endosome approximately 1.5 μ wide, could be distinguished. No nucleus was discernible in captives whose contents had been largely expropriated (FIG. 3, *C–E*). Many hyphae or portions of mycelium that had progressed to the production of chlamydospores (FIG. 3, *F–J*) were found beset with numerous empty membranous pellicles—evidence that capture of animals had earlier proceeded very abundantly. A single captured amoeba that was surrounded by a layer of stiffened ectoplasm about 0.4 μ thick (FIG. 3, *E*) clearly belonged to a species different from the amoeba with thin membranous pellicle which commonly served as prey.

Cystopage sacciformis most closely resembles the rhizopod-capturing *C. subtilis* Drechsler (**1941**) in the width of its mycelial filaments and in the dimensions of its chlamydospores, while in the positional relation of chlamydospore to mycelium it appears most nearly like the much more robust nematode-capturing *C. lateralis* Drechsler (**1941**). Its chlamy-

variations in size and shape. *B*, Detached conidia, *a–e*, of an *Acaulopage* obtained in a maize-meal-agar plate culture on which had been planted a small quantity of decaying potato-vine detritus gathered in western Maryland in October 1934. *C*, An *Acaulopage* obtained in a maize-meal-agar plate culture that had been planted with a small quantity of leaf mold taken from woods in northern Wisconsin, on September 10, 1938; *a*, portion of hypha and immature conidium; *b–d*, detached conidia. *D*, An *Acaulopage* obtained in a maize-meal-agar plate culture that had been planted with a small quantity of leaf detritus taken from woods in central North Carolina, on November 30, 1946; *a*, portion of hypha with 4 denuded sterigmata; *b*, detached conidium. *E*, Portion of mycelium, *a*, of *Stylopage rhynchospora* (Wisconsin strain) from which arises a conidiophore, *b*, bearing a young conidium, *c*, with widened beak. (Owing to lack of space *b* is shown in parts whose proper connection is indicated by broken lines.) *F*, Detached conidium of *S. rhynchospora* (Wisconsin strain) still continuous with its distended beak. *G*, Detached conidia of *S. rhynchospora* (Wisconsin strain), *a–h*, showing wide beaks delimited by a basal membrane. *H*, Two detached conidia of *S. rhynchospora* (Wisconsin strain) that are stuck together by their beaks. *I*, Wide-beaked conidium, *a*, of *S. rhynchospora* (Wisconsin strain), from which has been sent up a sporangiophore, *b*, bearing an immature conidium, *c*, with perceptibly distended beak.

dospores originate from the slender hyphae as pouch-like lateral protuberances (FIG. 3, *F–J*), which grow slowly as they receive materials from the adjacent portions of parent filament. When the parent filament has been emptied the individual protuberance is usually delimited at its narrow attachment as a sessile bursiform chlamydospore (FIG. 3, *K*). In any random assortment of chlamydospores (FIG. 3, *L–O; P, a–z; Q, a–g;* FIG. 4, *A, a–z*) rather wide variations with respect to size, shape and basal attachment are observable. Some few spores that are delimited by 2 cross-walls in the parent filament (FIG. 3, *P, b;* FIG. 4, *A, b*) are almost certainly somewhat immature, being most probably destined to undergo further withdrawal of protoplasm from the basal end and thereupon to be delimited anew by a single retaining wall (FIG. 3, *L; P, f, k, m, n, p*). Sometimes withdrawal of contents continues after the parent hypha has been emptied, so that eventually the chlamydospore is found attached to the end of a widening empty stalk (FIG. 3, *P, d, e, l, x;* FIG. 4, *A, i, z*) usually only a few microns long. In exceptional instances a chlamydospore is borne terminally on a lateral branch as much as 20 μ long (FIG. 4, *A, s*), thereby providing some little parallelism with the robust nematode-capturing *C. cladospora* Drechsler (**1957**). As its broad distal portion is often extended unsymmetrically and at a noticeable angle with its narrower proximal portion, the chlamydospore often has a saclike shape which is intended to be signalized in the specific epithet. Mesially intercalary chlamydospores of symmetrically globose or ellipsoidal shape like those of the robust nematode-capturing *C. intercalaris* Drechsler (**1945**) and of the 2 amoeba-capturing congeners *C. ellipsospora* Drechsler (**1955**) and *C. sphaerospora* Drechsler (**1955**) have never been observed in *C. sacciformis*.

Acaulopage tenuicornis sp. nov.

Mycelio exiguo; hyphis incoloratis, filiformibus, p[a]ce vel mediocriter ramosis, interdum aliquantum pravis, plerumque 1–1.7 μ crassis, ad *Amoebas* inhaerentibus, ita animalia capientibus, pelliculam cujusque captivi perforantibus, haustorium intrudentibus quod protoplasma exhaurit; haustorio pediculato, pediculo ejus vulgo 3–5 μ longo et 1–1.3 μ lato 4–20 ramos divaricatos 1–11 μ longos 1–1.5 μ latos ferente; sterigmatibus erectis, saepius 2–3 μ longis, basi 1 μ latis, apice circa 0.6 μ latis; conidiis ex toto plerumque 23–43 μ longis, in inferiori viventi cellula et superiori vacua appendice constantibus—viventi cellula incolorata, elongato-fusiformi, plerumque 13–21 μ longa et 2.8–3.6 μ lata—vacua appendice filiformi vel subuliformi, 10–22 μ longa, basi 0.8–1.3 μ lata, prope apicem 0.4–0.8 μ lata.

Amoebam saepius 20–30 μ latam capiens consumensque habitat in foliis arborum putrescentibus prope Durango, Colorado. Typus: Figura 5.

Mycelium scanty; hyphae colorless, filamentous, sparingly or moderately branched, sometimes rather crooked, mostly 1 to 1.7 μ wide, adhering to amoebae and thus capturing the animals, then perforating the pellicle of each captive and intruding a haustorium to appropriate the fleshy contents; haustorium pedicellate, consisting of a pedicel 3 to 5 μ long and 1 to 1.3 μ wide and of 4 to 20 assimilative branches that result from successive dichotomies and measure mostly 1 to 11 μ in length and 1 to 1.5 μ in greatest width; sterigmata erect, 2 to 3 μ long, 1 μ wide at the base, about 0.6 μ wide near the tip; conidium mostly 23 to 43 μ in total length, composed of a proximal living cell and a distal empty appendage—the living cell being usually elongate-fusiform and measuring 13 to 21 μ in length and 2.8 to 3.6 μ in greatest width—the empty appendage being usually filamentous or awl-shaped and measuring 10 to 22 μ in length, 0.8 to 1.3 μ in basal width, and 0.4 to 0.8 μ in apical width.

Acaulopage tenuicornis developed in a maize-meal-agar plate culture which after being overgrown by *Pythium ultimum* Trow had been further planted with a few pinches of leaf mold taken from woods in southwestern Colorado on July 3, 1958. Its sparse mycelium looked much like that produced by many other members of the family. The amoeba (FIG. 5, *A–E: a; F, a–c*) on which the fungus evidently subsisted to the exclusion of all other food materials was enveloped by a pellicle, if such it can be called, consisting of a somewhat stiffened mantle of ectoplasm about 0.4 μ thick. Directly under the pellicle the main mass of ectoplasm formed a clear layer, mostly 2 to 5 μ thick, which surrounded a large central region occupied by a massive aggregation of globules. The pedicel of the haustorium was always clearly revealed as it traversed the transparent ectoplasmic layer, whereas the assimilative branches, being deeply inserted into the nearly opaque mass of globules, were largely hidden from view during the earlier stages of attack (FIG. 5, *A–C: a*). Later, as the animal was progressively expropriated of its contents, the assimilative branches became increasingly visible (FIG. 5, *D, a; E, a; F, b, c*). The diminished contents in some instances disclosed also a globose body that seemed interpretable as the animal's nucleus (FIG. 5, *D, n; F, n*). During the final stages of attack the haustorium commonly remained recognizable for some time after any globose body that could be interpretated as a protozoan nucleus had vanished (FIG. 5, *E, a; F, c*).

In my culture *Acaulopage tenuicornis* showed no sexual reproduction and displayed asexual reproduction only rather sparingly. Conidial development in the fungus evidently takes place much as in the congeneric species I described earlier (Drechsler, **1936**, **1922**, **1948**) under

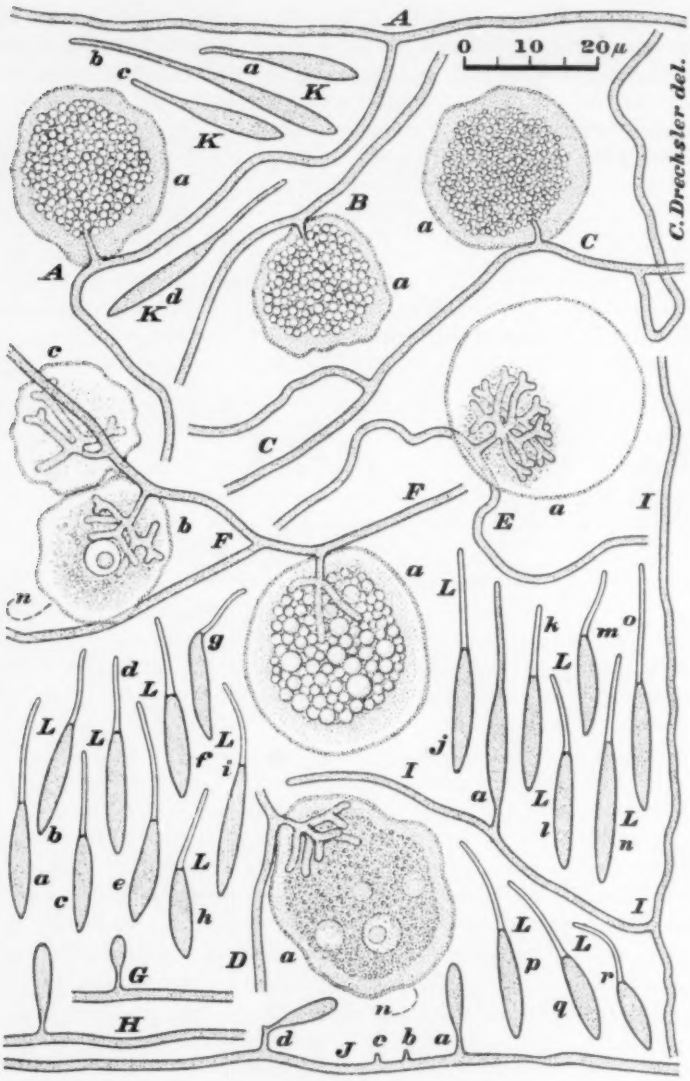

FIG. 5.

FIG. 5. *Acaulopage tenuicornis*, drawn at a uniform magnification with the aid of a camera lucida; × 1000. *A–C*, Portions of hyphae, simple or branched, each with a rather newly captured amoeba, *a*, into which has been extended a

the binomials *A. cercospora, A. gomphoclada* and *A. gyrinodes.* Here and there a prostrate hypha puts forth a slender outgrowth which after attaining a length of 2 to 3 μ elongates with some increase in width (FIG. 5, *G; H; I, a; J, a*), thereby forming a strobiloid enlargement. Through further elongation at reduced width a narrow filamentous beak approximately equal in length to the strobiloid distention is produced distally (FIG. 5, *I, a*). In some instances the distended part is now delimited proximally by a cross-wall and while still continuous with the filamentous beak is abjointed as a conidium (FIG. 5, *K, a–d*). Usually, however, abjunction is delayed until after the filamentous beak has been evacuated of all protoplasmic contents, so that the detached spore is commonly found composed of an elongate-fusiform living cell and an empty tubular appendage (FIG. 5, *L, a–r*). When the narrow basal outgrowth, or sterigma, has been denuded (FIG. 5, *J, b, c*) through abjunction of a first conidium, it may give rise, often somewhat obliquely from a subapical position, to a second conidium (FIG. 5, *J, d*).

Acaulopage tenuicornis differs conspicuously from *A. cercospora* in the greater length and correspondingly greater size of the living cell forming the basal component of its conidium. It does not seem likely that this greater size can be attributed to better conditions for development, for in the culture from which was taken the material used in preparing the description of *A. cercospora,* conidia were produced much more abundantly than in my culture of *A. tenuicornis.* The haustorium of *A. tenuicornis* with its more numerous dichotomies and longer assimilative branches would seem likewise to distinguish the fungus from *A. cercospora.* With respect to abundance of haustorial branching *A. tenuicornis* apparently also exceeds *A. gomphoclada,* although it is separated from that species more obviously by the greater width of its

haustorium whereof only the pedicel is visible, since the assimilative branches are concealed in the strongly globuliferous endoplasm. *D, E,* Unbranched portions of hypha, each with a captured amoeba, *a,* within which the haustorium—and in *D* also the animal's nucleus, *n*—has become visible, owing to disappearance of globulose materials. *F,* Branched hypha with 3 captured amoebae, *a–c;* in captive *a* the haustorium is still partly concealed by globulose contents, while in captives *b* and *c* it is wholly visible; in *b* the animal's nucleus, *n,* is also shown. *G, H,* Unbranched portions of hyphae, each with an elongating young conidium. *I,* Mycelial hypha with a branch on which a full grown conidium, *a,* is shown still continuous with its sterigma and its appendage. *J,* Prostrate hypha with 4 sterigmata, *a–d; a* supports an actively growing conidium, *b* and *c* are denuded, *d* is producing a second conidium after having been denuded of a first conidium. *K,* Detached conidia, *a–d,* each with its appendage still filled with protoplasm. *L,* Fully developed conidia, *a–r,* showing variations in size and shape of the living cell and of the empty appendage.

conidia. From *A. gyrinodes*, in which decidedly delicate hyphae give rise to robust conidia, *A. tenuicornis*, as also *A. cercospora* and *A. gomphoclada*, differs in the greater width of its mycelial filaments as well as in the lesser width and lesser total length of its asexual spores.

An *Acaulopage* rather closely resembling *A. tenuicornis* was observed 25 years ago in some maize-meal-agar plate cultures that had been planted with a small quantity of potato (*Solanum tuberosum* L.) detritus collected in western Maryland in October 1934. Its conidia (FIG. 4, *B*, *a–e*) would seem to have been somewhat narrower than those of *A. tenuicornis* as they usually measured 2.2 to 2.7 μ in greatest width. Spore dimensions more accurately corresponding to those of *A. tenuicornis* were noted in an *Acaulopage* (FIG. 4, *C*, *a–d*) that developed several years later in a Petri plate culture to which had been added some leaf mold taken from woods near Park Falls, Wisconsin, on September 10, 1938, though the detached conidia here showed a minute basal protrusion, usually about 0.7 μ long and 0.5 μ wide. An *Acaulopage* not greatly different from *A. tenuicornis* or *A. cercospora*, though probably not identical with either of these species, was obtained from leaf mold collected near Greensboro, North Carolina, on November 30, 1946. On erect tapering sterigmata (FIG. 4, *D*, *a*) mostly 3 to 8 μ long and 1 μ wide at the base, this fungus bore conidia (FIG. 4, *D*, *b*) furnished with a minute empty stipe at the proximal end as well as with an empty tubular appendage at the distal end. In these conidia the fusiform living cell measured about 10 μ in length and 2 μ in greatest width, while the distal appendage was usually about 10 μ long and 0.8 μ wide.

ACAULOPAGE ISCHNOSPORA Drechsler var. **pleacra** var. nov.

A typo differt praecipue conidiis in apice non inanitibus sed protoplasmatis omnino repletis.

Amoebam vulgo copiose globuliferam, pellicula cartilaginea circa 0.4 μ crassa circumdantem, plerumque 20–35 μ latam capiens consumensque habitat in foliis arborum putrescentibus prope Willards, Maryland. Typus varietatis: Figura 6.

Differing from the type of the species in that the conidia are not empty at the tip but are filled throughout with protoplasm.

The fungus here presented as a variety of *Acaulopage ischnospora* Drechsler (**1947a**) developed in a maize-meal-agar plate culture which after being overgrown by mycelium of *Pythium ultimum* had been further planted with a small quantity of leaf mold kindly collected by W. F. Jeffers in woods near Willards, Maryland, on March 7, 1953. It subsisted through capture of an amoeba that resembled neither of the 2

animals earlier found undergoing destruction by *Acaulopage ischnospora*, but instead strongly resembled the animal habitually consumed by *Acaulopage tenuicornis*. Captured specimens of the amoeba in question (FIG. 6, *A–D*) were surrounded by a stiffened mantle of ectoplasm about 0.4 μ thick rather than by a thinner yet apparently much firmer pellicle of the membranous type present in many terricolous rhizopods. A large central region of the protoplasmic interior was occupied by a dense aggregation of globules in which the nucleus (FIG. 6, *S*) was usually concealed. After intrusion of a haustorium into the captive the closely arranged globules also concealed largely, if not wholly, the assimilative branches extending between them, though the slender pedicel traversing the clear layer of soft ectoplasm directly under the stiffened mantle always remained in plain sight. Later when the globules had very largely disappeared—the substance composing them having presumably been appropriated by the fungus—the assimilative branches emerged into view (FIG. 6, *E, a, b*). Soon afterwards in most instances the animal's mantle disintegrated, so that the haustorium, together often with some residual globules enmeshed between its branches (FIG. 6, *F, G*), was bared to external agents. Apparently the haustorium did not well endure exposure. In a short time its contents usually assumed the dense, somewhat opaque texture often expressive of protoplasmic degeneration in the Zoopagaceae.

Asexual reproduction in *Acaulopage ischnospora* var. *pleacra* takes place (FIG. 6, *H; I, a, b*) much as in the several other known congeners with somewhat filiform conidia, including not only *A. ischnospora* but also the species I described (Drechsler **1935b**, **1941**, **1948**) under the binomials *A. rhicnospora*, *A. stenospora* and *A. baculispora*. With respect to the dimensions of its sterigmata (FIG. 6, *I, a–d*) as well as with respect to the general shape, greatest width, and total length of its basally delimited (FIG. 6, *I, e, f*) or its detached conidia (FIG. 6, *J, a–g*), it agrees well with the description of *A. ischnospora*. Further it shows parallelism with *A. ischnospora* in accomplishing sexual reproduction usually through union of a branch given off by a mycelial filament (FIG. 6, *K–M: a*) with a germ hypha extended from a conidium (FIG. 6, *K–M: b*). This parallelism is sustained well by resemblances pertaining to the shape, dimensions, and internal structure of the resulting zygospores (FIG. 6, *N–R*).

Although the fungus from southeastern Maryland was examined extensively in an undisturbed condition under a dry objective, and also in covered mounts under an immersion objective of higher magnification, no instance could be found where a conidium terminated in an empty

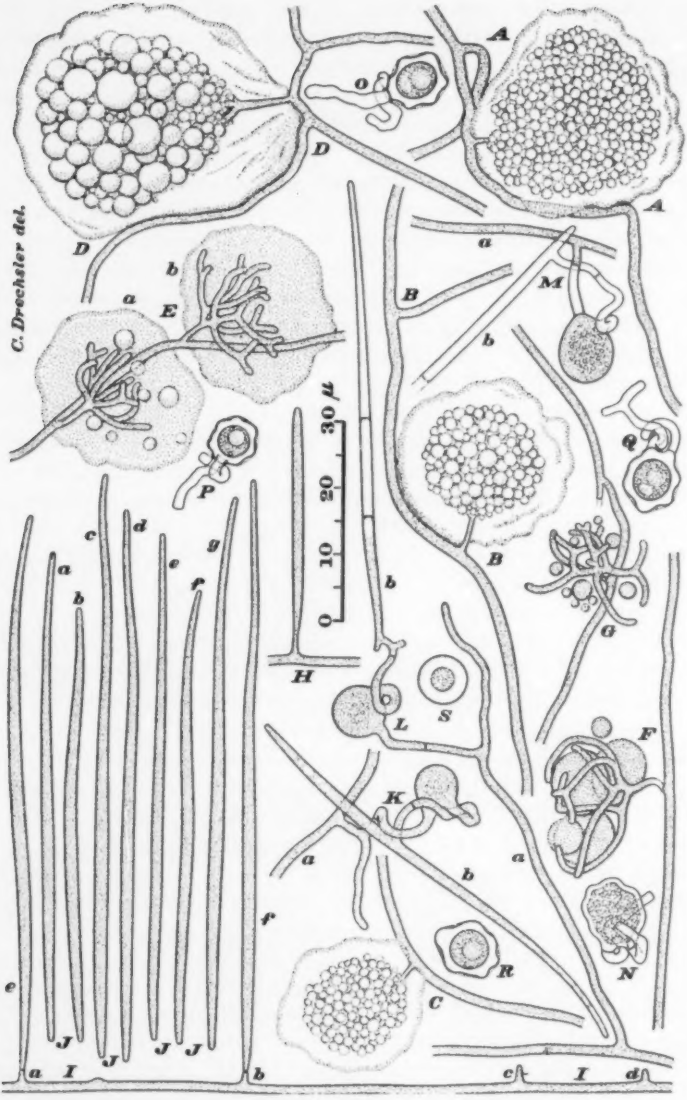

FIG. 6.

FIG. 6. *Acaulopage ischnospora* var. *pleacra*, drawn at a uniform magnification with the aid of a camera lucida; × 1000. *A–D*, Portions of hyphae, each with

tubular appendage. Withdrawal of protoplasmic contents from any portion of a conidium was observed only when germination took place, whether by emission of a vegetative filament or of a sexual hypha. Designation of the fungus by a term compounded of two words (πλέος, ἄκρα) meaning "full" and "tip," respectively, is intended to signalize its chief distinguishing character. Duddington (**1951**), who obtained from bryophytes in Devon, England, some material of *A. ischnospora* in which the conidia showed no distal appendage, recently suggested that the British and American strains might represent different varieties (Duddington **1956**). The Devon strain might be identical with the fungus from southeastern Maryland, though in drawings by Duddington (**1951**, p. 205, fig. 6, a) the conidia of the former appear of nearly uniform width from base to tip, whereas the conidia of the latter commonly taper in noticeable measure toward both ends.

The disintegration of the animals captured by *Acaulopage ischnospora* var. *pleacra* before their contents have been fully assimilated is probably attributable more to the undurable character of their ectoplasmic mantle than to any peculiarity of the fungus. Lack of a protective layer substantial enough to be visible under the microscope is associated with similarly early disintegration of the proteomyxan rhizopod attacked by *Acaulopage crobylospora* Drechsler (**1947a**) and of the amoeba captured by *Zoopage pachyblasta* Drechsler (**1947b**). Premature disintegration is ordinarily not observed when amoebae provided with a membranous pellicle are attacked by any predacious or parasitic member of the Zoopagaceae. Since in instances of such attack the very slender infection tube continues throughout the period of expropriation to occupy snugly the small hole it made in penetrating the pellicle, and since, further, the seam between pellicle and tube is well calked with adhesive

a captured amoeba invaded by a haustorium whose branches are largely concealed in the strongly globuliferous endoplasm. *E*, Portion of hypha with 2 captured amoebae, *a–b*, both of them largely expropriated of their contents, so that the intruded haustorium is wholly visible. *F*, *G*, Portions of hyphae, each with a haustorium whose branches extend between some globules left after the ectoplasmic mantle of the captured amoeba disintegrated. *H*, Portion of prostrate hypha showing a pedicel with a half-grown conidium. *I*, Portion of procumbent hypha with 4 sterigmata, *a–d*, of which two, *a–b*, support basally delimited conidia, *e–f*. *J*, Detached conidia, *a–g*, showing usual variations in size and shape. *K–M*, Young sexual reproductive units showing different stages in growth of the fusion cell and origin of each unit from conjugation of a branch supplied by a mycelial hypha, *a*, with a germ hypha extended from a conidium, *b*. *N*, Immature zygospore with attached membranes of the gametangia. *O–R*, Mature zygospores. *S*, Nucleus of the amoeba commonly serving as prey.

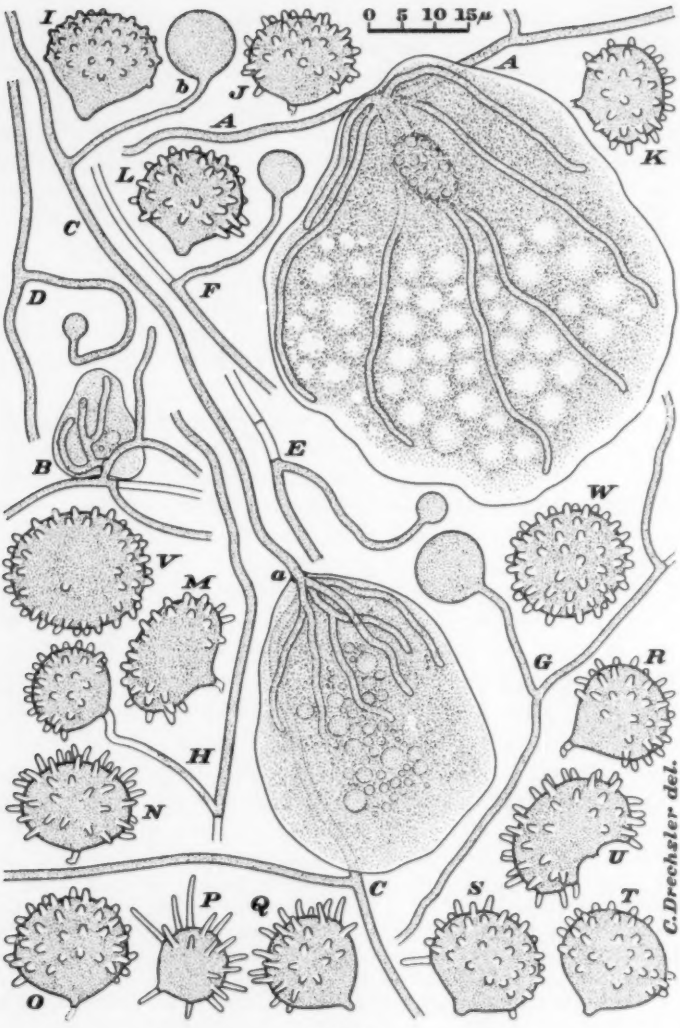

FIG. 7.

FIG. 7. *Acaulopage trachyspora*, drawn at a uniform magnification with the aid of a camera lucida; ×1000. *A, B*, Branched portions of hyphae, from each of which haustorial branches have been extended into a captured amoeba. *C*, Portion of prostrate mycelium; *a*, captured amoeba invaded by haustorial branches; *b*, branch bearing an immature conidium. *D–G*, Portions of hyphae, each with a

material, all alien microorganisms are effectively excluded from the interior of the animal.

Acaulopage trachyspora sp. nov. (*τραχύς*, rough; *σπορά*, seed)

Mycelio exiguo; hyphis incoloratis, filiformibus, parce ramosis, primo continuis, vulgo 1–1.8 *μ* latis, submersis vel procumbentibus, ad animalia minuta inhaerentibus, pelliculam cujusque capti perforantibus, haustorium intrudentibus quod protoplasma exhaurit; haustorio in 2–8 ramulis divaricatis 10–60 *μ* longis et circa 1.2 *μ* latis consistente; ramulis fertilibus ex hyphis procumbentibus hic illic oriundis, vulgo 15–30 *μ* longis, 1–1.2 *μ* crassis, in parte ultima saepe in aerem ascendentibus, apice unum conidium ferentibus; conidiis incoloratis, globosis vel turbinatis vel applanato-ellipsoideis, interdum latioribus quam crassis, aliquando sursum bifidis, 12–18 *μ* longis, 11–27 *μ* latis, basi interdum stipitatis, prope basim semper levibus, medio saepius appendicibus ornatis, apice vulgo appendicibus ornatis; appendicibus 20–90 omnino, in maturitate vulgo vacuis, plerumque 0.6–8 *μ* longis, basi 1–1.2 *μ* latis, sursum attenuatis, apice rotundatis, itaque verruciformibus vel digitiformibus.

Amoebam vulgo 15–60 *μ* latam capiens et consumens habitat in foliis graminis putrescentibus prope Kimberly, Idaho. Typus: Figurae 7 et 8.

Mycelium scanty; hyphae colorless, filamentous, sparingly branched, at first continuous, commonly 1 to 1.8 *μ* wide, submerged or prostrate, capturing minute animals by adhesion, then penetrating the pellicle of each captive and extending into it a haustorium that appropriates the protoplasmic contents; haustorium consisting of 2 to 8 divergent branches 10 to 60 *μ* long and about 1.2 *μ* wide; some procumbent mycelial hyphae giving off prostrate or distally ascending fertile branches 15 to 30 *μ* long and 1 to 1.2 *μ* wide, which each produces a conidium at the tip; conidia colorless, globose or turbinate or oblate ellipsoidal, distally sometimes wider than thick, now and then distally bilobate, mostly 12 to 18 *μ* long, 11 to 27 *μ* wide, sometimes noticeably stipitate, smooth around the basal attachment, very often furnished with protuberances around the equator, nearly always beset with protuberances distally; protuberances 20 to 90 in total number, usually empty at maturity, mostly 0.6 to 8 *μ* long and 1 to 1.2 *μ* wide at the base, slightly tapering upward, rounded at the tip, hence often wartlike or finger-shaped.

Acaulopage trachyspora first came under observation late in January 1957 in some maize-meal-agar plate cultures which after being overgrown by *Pythium debaryanum* Hesse had been further planted with small quantities of grass detritus kindly gathered by W. J. Zaumeyer near Kimberly, Idaho, in August 1956. Although during the 5 months

branch bearing a young conidium. *H*, Portion of hypha with a branch bearing a mature conidium. *I–U*, Detached conidia in lateral view, showing usual variations in size and general shape, as well as in number and size of the protuberances. *V*, Detached conidium in bottom view. *W*, Detached conidium in top view.

following its collection the detritus had received only ordinary care, having been stored in an air-dry state at room temperatures between 20 and 35°C, the fungus grew extensively over the agar substratum during the first week after the plantings were made. Prompt and extensive development of the fungus likewise ensued in April 1958, when small pinches of the detritus, now 20 months old, were again superadded to newly prepared maize-meal-agar plate cultures of *P. debaryanum*. Presumably because this vigorous early development must have greatly reduced the supply of living protozoans available for capture, vegetative growth and asexual reproduction of the fungus proceeded only slowly after the detritus had been on the cultures for 15 days.

The mycelium of *Acaulopage trachyspora* is of moderate coarseness in comparison with that of other members of the Zoopagaceae subsisting through capture of rhizopods. As a rule the hyphae (FIG. 7, *A–H;* FIG. 8, *A–E*) are little given to abrupt variations in width. Branches for the most part are of approximately the same diameter as the hyphae from which they arise. Captured animals are found attached to the hyphae by means of adhesive secretion. In the area of contact the hypha puts forth a protuberance that pushes its way through the small deposit of yellow adhesive substance, penetrates the animal's membranous pellicle and then ramifies to form the bush-like haustorium. The haustorial branches in some instances (FIG. 7, *A, B;* FIG. 8, *D, a*) extend virtually the entire length of the captive, but in other instances they cease elongating when they reach somewhat more than halfway through the rhizopod (FIG. 7, *C, a;* FIG. 8, *A, C*). The animal's murky protoplasm, in which it has not been possible to distinguish a nucleus clearly, now diminishes steadily (FIG. 8, *A; C; D, a, b*). Eventually the contents of the haustorial branches retreat backward into the parent hypha, and the empty tubular membranes, like the depleted pellicle, gradually vanish from sight.

The Petri plate cultures to which grass detritus was added showed, distributed over the surface of the substratum 8 days later, some fully mature conidia of *Acaulopage trachyspora* as well as some immature conidia in different formative stages. The young conidium first becomes recognizable as a smooth globose swelling (FIG. 7, *D, E*) at the tip of a lateral branch usually 20 to 30 μ long and about 1.2 μ wide. A proximal portion of the branch is commonly prostrate on the substratum, whereas the distal portion often ascends slopingly into the air. As the terminal swelling gradually expands (FIG. 7, *C, b; G*) it usually increases somewhat more in width than in length, and consequently tends to assume a noticeably flattened or oblate ellipsoidal shape. In the final

stage of growth (FIG. 8, *E*) it puts forth rounded or tapering protuberances, which ordinarily soon become emptied of their protoplasmic contents (FIG. 7, *H*). A wall is laid down near the base of the globose body. The conidium thus delimited may become detached on slight disturbance, such as is often provided by the jostling of a nematode; or it may be set free less abruptly through disintegration of the membranous envelopes of the evacuated supporting branch.

In lateral view the globose or oblate ellipsoidal shape usual among detached conidia (FIG. 7, *I–U;* FIG. 8, *F–V*) is often found modified very noticeably by the presence of a basal protrusion (FIG. 7, *I, L, M, R–T;* FIG. 8, *F, G, P–S*), so that a somewhat turbinate profile is presented. Now and then, however, the basal protrusion juts from the middle of a concave basal contour (FIG. 7, *U;* FIG. 8, *T, U*). In such instances, especially where the distal contour is also perceptibly concave (FIG. 8, *V*), the conidium may present a distinctly bilobate outline when it is seen from above (FIG. 8, *W*) rather than the circular or elliptical outline usually shown in bottom (FIG. 7, *V*) or in top (FIG. 7, *W*) view. Whether the proximal contour of the conidium is convex or concave, a circumbasal area of variable extent generally remains smooth. In the equatorial and subequatorial regions protuberances are distributed somewhat less consistently than in the apical region. Although the protuberances vary little in width proximally, they vary conspicuously in length. Thus, while some few conidia (FIG. 7, *I*) bear only small wartlike excrescences 0.6 to 0.8 μ in length, some few others (FIG. 7, *P*) bristle with tapering digitations 5 to 8 μ long. A range in length from 1 to 2.5 μ may perhaps be held usual for protuberances in the species. In most individual conidia the protuberances are of more nearly uniform length than this range might suggest, but some conidia (FIG. 8, *J, U*), again, bear a few long digitations interspersed among a larger number of protuberances less than 2.5 μ in length. Of the 20 to 90 protuberances present on a conidium 15 to 60 are exposed to view in the upper aspect and along the periphery.

Because of its tapering conidial protuberances *Acaulopage trachyspora* appears readily distinguishable from the several congeneric forms I described (Drechsler **1942**, **1946**, **1955**) under the binomials *A. lasiospora*, *A. hystricospora* and *A. dactylospora*, in which the conidia are beset with protuberances of about equal width from base to tip. It is separated even more decisively from *A. dasyspora* Drechsler (**1955**), in which the conidia bear protuberances that commonly are expanded at the tip. In *A. acanthospora* Drechsler (**1938**) and *A. acanthospora* var. *magna* Juniper (**1953**) tapering protuberances are borne on somewhat

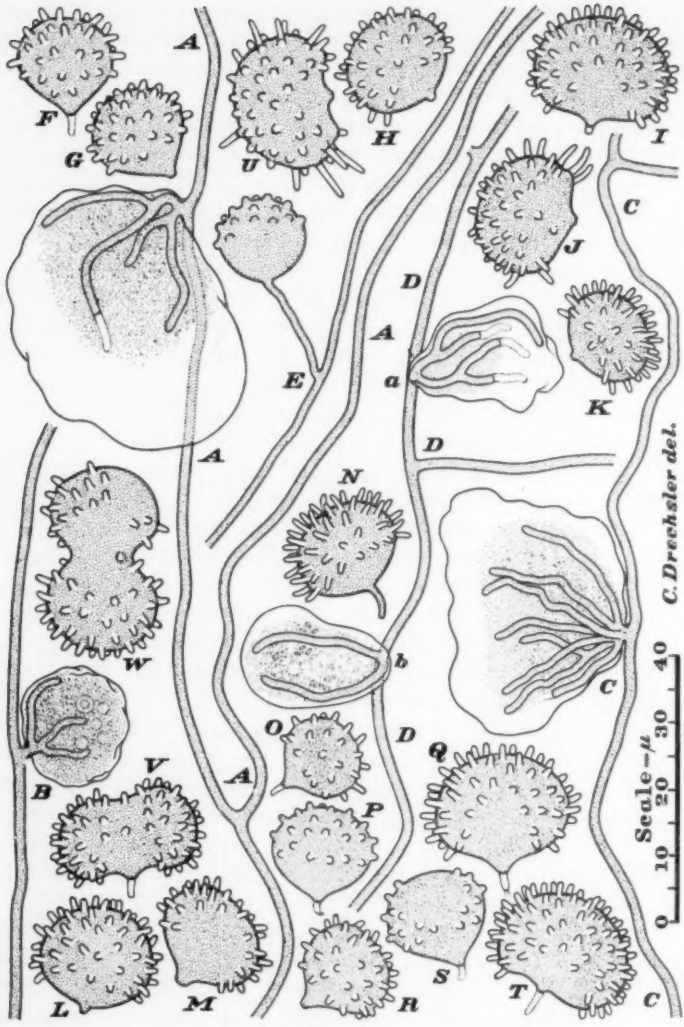

FIG. 8.

FIG. 8. *Acaulopage trachyspora*, drawn at a uniform magnification with the aid of a camera lucida; × 1000. *A–C*, Portions of mycelium, each with a captured amoeba into which a haustorium has been extended. *D*, Portion of mycelium with 2 captured amoebae, *a–b*, each occupied by a haustorium. *E*, Portion of hypha with a branch bearing an immature conidium. *F–V*, Detached conidia in lateral view,

turbinate conidia, which generally are longer than wide, whereas in *A. trachyspora* such protuberances are borne on larger conidia that usually are noticeably—and, indeed, sometimes are conspicuously—wider than long.

Cochlonema cerasphorum sp. nov.

Hyphae assumentes incoloratae, continuae, vulgo 25–65 μ longae, 4.5–8 μ latae, in axe vulgo simplices, plerumque semel interdum paene bis in spiram volutae, prope originem 1 vel 2 hyphas genitabiles et aliquot (vulgo 1–4) cornua sterilia 4–9 μ longa, 2–3 μ lata emittentes; hyphae genitabiles basi 2–3 μ crassae, protinus usque 1.2–1.6 μ leniter attenuatae, pelliculam animalis perforantibus, extra simplices vel parce ramosae, mox in 1–3 catenulas conidiorum ascendentes abeuntes; conidia incolorata, cylindrata vel elongato-ellipsoidea vel fusiformia, raro ramosa, plerumque minuta verrucosa, 5–18 μ longa, 1.7–2.5 μ crassa, 5–30 in quaque catenula.

Amoebam saepius 20–35 μ latam interficiens habitat in foliis arborum putrescentibus prope Park Falls, Wisconsin. Typus: Figura 9.

Assimilative hyphae colorless, continuous, commonly 25 to 65 μ long, 4.5 to 8 μ wide, unbranched with respect to the main axis, convolved into a flat spiral of 1 to $1\frac{3}{4}$ turns, from the proximal end giving rise to 1 or 2 conidiiferous hyphae and from positions nearby—especially from positions opposite to conidiiferous hyphae—putting forth 1 to 4 sterile horn-shaped projections mostly 4 to 9 μ long and 2 to 3 μ wide; conidiiferous hyphae 2 to 3 μ wide at the base, tapering immediately though somewhat gradually to a width of 1.2 to 1.6 μ, perforating the animal's pellicle, at maturity terminating externally in 1 to 3 ascending conidial chains; conidia colorless, cylindrical or elongate-ellipsoidal or spindle-shaped, rarely branched, usually minutely verrucose, 5 to 18 μ long, 1.7 to 2.5 μ in greatest width, formed in numbers from 5 to 30 in each chain.

Cochlonema cerasphorum was found in some maize-meal-agar plate cultures which after being overgrown with *Pythium* mycelium had been further planted with small portions of leaf mold collected in northern Wisconsin on November 10, 1957. All individuals of the amoeba on which the fungus subsisted showed during the earlier and somewhat more advanced stages of attack (FIG. 9, *A–F*) a thin delicately rippled pellicle surrounding rather transparent, finely granular protoplasm. In this protoplasm was clearly visible an elongated ellipsoidal nucleus, mostly 5 to 9 μ long and 4 to 6 μ wide, that contained about a dozen flattened ellipsoidal bodies in peripheral positions (FIG. 9, *A–F: n*). From similarities in nuclear structure the host animal seemed to be

showing usual variations in size and general shape as well as in number and size of the protuberances. *W*, Detached bilobate conidium in top view.

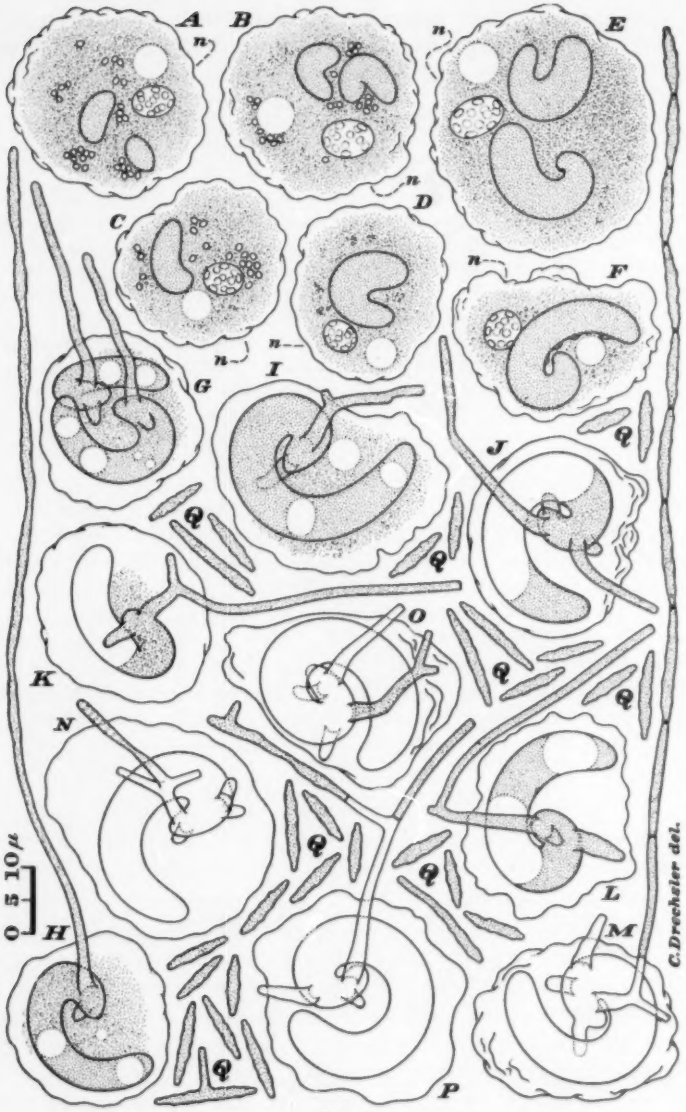

FIG. 9.

FIG. 9. *Cochlonema cerasphorum*, drawn at a uniform magnification with the aid of a camera lucida; × 1000. *A–F*, Specimens of host amoeba containing one

conspecific with the amoeba which was earlier found attacked by *Zoopage thamnospira* Drechsler (**1938**) and *Acaulopage tetraceros* Drechsler (**1942**), and which, together with an amoeba of different nuclear make-up, was reported as being destroyed by both *Acaulopage ischnospora* and *Zoopage virgispora* Drechsler (**1947b**). Invasion of the animal was accomplished usually by one (FIG. 9, *C, D, F, H–P*) or two (FIG. 9, *A, B, E, G*) assimilative hyphae. In an early stage of development each of these hyphae consisted of an elongated ellipsoidal cell (FIG. 9, *A*) to which the empty membrane of the parent conidium was never seen attached. On attaining a length of 10 μ the cell became noticeably bent (FIG. 9, *B, C*). For some time its further growth was accompanied by rather pronounced curvature. After a flat spiral of approximately one turn had been described, elongation continued with diminished curvature, so that in many instances the fully developed vegetative hyphae, or thallus, came to resemble an obese Arabic numeral 6 (FIG. 9, *F, L, O, M*) or the reverse of that figure (FIG. 9, *I, J*). Dichotomous branching, frequent in some congeneric species, was not observed in any assimilative hyphae of *C. cerasphorum*.

When the infected animal had become disabled from loss of protoplasm—a condition usually associated with partial or complete obliteration of the nucleus—the fungus initiated asexual reproduction. Each spiral thallus extended a conidiiferous hypha from a position 2 to 5 μ from its proximal end (FIG. 9, *G–I*). In instances where 2 conidiiferous hyphae were produced, the second one was put forth more distally, usually being extended from a position 6 to 8 μ from the proximal end (FIG. 9, *J, O*). The conidiiferous hyphae resembled those of *Cochlonema euryblastum* Drechsler (**1942**) in tapering rather markedly from an unusually wide basal attachment. Where the spiral assimilative thallus of *C. cerasphorum* was oriented flatwise in a horizontal plane, the conidiiferous hyphae were usually found originating from positions

(*C, D, F*) or two (*A, B, E*) assimilative hyphae; *n*, host nucleus. *G*, Host amoeba containing 2 assimilative thalli, each with a sterile spur and an elongating conidiiferous hypha. *H*, Host amoeba occupied by one assimilative thallus that bears a sterile spur and an elongating conidiiferous hypha. *I–L*, Host pellicles, each occupied by a partly evacuated assimilative thallus from which have been extended one (*I, K*) or two (*J, L*) sterile spurs and one (*I, K, L*) or two (*J*) conidiiferous hyphae; the conidiiferous hyphae being shown without the conidial chains in which they terminated. *M*, Host pellicle occupied by an empty assimilative thallus which is furnished with 2 sterile spurs and 2 conidiiferous hyphae; the more proximal of the hyphae bore 2 conidial chains, of which one is shown. *N–P*, Host pellicles occupied by solitary empty assimilative thalli, showing further variations in attachment of empty spurs and conidiiferous hyphae. *Q*, Detached conidia, showing usual variations in size and shape.

on the upper aspect of the coil, not as in *C. curyblastum* from positions along the outer profile of the coil.

Cochlonema cerasphorum is distinguished from all known congeneric species by the short sterile spurs that it puts forth concomitantly with the conidiiferous hyphae. Usually, though not invariably, the individual spur arises in a position opposite a conidiiferous hypha (FIG. 9, *G–P*) and extends in an opposite direction. While in many thalli a single conidiiferous hypha is accompanied by a single sterile spur (FIG. 9, *G, H, I, K*), or 2 conidiiferous hyphae are associated with 2 spurs (FIG. 9, *J, M, O*), such numerical equality is not maintained in thalli where a single conidiiferous hypha is flanked by two (FIG. 9, *L, P*) or three (FIG. 9, *N*) sterile spurs. The spurs are commonly of the same width as the conidiiferous hyphae and taper in approximately the same measure. Like the assimilative thalli they eventually yield up their protoplasmic contents without forming any transverse walls to mark successive stages in their evacuation. Their somewhat thickened, slightly verrucose envelopes long remain visible as tubular appendages (FIG. 9, *M–P*) which jut from the proximal portion of the thallodic membrane like sturdy horns, thereby suggesting as epithet for the fungus a term (κερασφόρον) meaning "horn-bearing."

From resemblance in position and attachment the sterile spurs would seem interpretable morphologically as conidiiferous hyphae of arrested development. They may have some little parallelism with the frustrated reproductive outgrowths commonly found in *Aplectosoma microsporum* Drechsler (**1951**) after exposure to microscopial examination. However, as they make their appearance regularly in cultures kept under conditions ordinarily prevailing in laboratories, it appears unlikely that their origin could be related to reduced air supply or to unusual illumination.

The aerial prolongations (FIG. 9, *H*) and aerial branches of the conidiiferous hyphae are converted by segmentation into conidial chains (FIG. 9, *M*). On slight disturbance the chains disintegrate, leaving detached spores (FIG. 9, *Q*) strewn about on the substratum, ready to infect new host animals.

STYLOPAGE RHYNCHOSPORA Drechsler (**1939**)

During the 20 years since it was described *Stylopage rhynchospora* has come under observation many times in cultures planted with decaying materials from localities in the eastern United States. Though it is now known to be distributed over a range of latitudes from Wisconsin to Florida, the fungus, nevertheless, has not developed very

frequently in my cultures. Marked departures from the morphology set forth in the original account have not appeared, though a somewhat aberrant tendency was noted in a culture planted with small pinches of garden refuse collected near Butternut, Wisconsin, on November 19, 1954. The mycelial hyphae (FIG. 4, *E, a*) in this culture measured up to 2.2 μ in width. They gave rise here and there to erect conidiophores (FIG. 4, *E, b*), each bearing a conidium (FIG. 4, *E, c*) terminating in a beak, which, instead of usually maintaining a uniform width to its rounded apex, most often was noticeably distended. At first the knob-like beak was without internal demarcation (FIG. 4, *F*), but in most detached conidia (FIG. 4, *G, a–h; H*) it contained no protoplasm and was delimited from the living cell below by a retaining wall. Germination of detached conidia (FIG. 4, *I, a*) took place frequently by production of a conidiophore (FIG. 4, *I, b*) bearing a secondary conidium (FIG. 4, *I, c*), which likewise was furnished with an expanded beak. As a similarly expanded beak is shown in an illustration of a secondary conidium given in the earlier account (Drechsler, **1939**, FIG. 2, *I*), the modification observed in the Wisconsin strain was apparently not wholly alien to the Virginia strain from which the species was described. Agreement was further evident in that some conidia of the Wisconsin strain were found sticking together by their beaks (FIG. 4, *H*). Although such cohesion is not abundantly displayed—Duddington (**1950**) saw no sign of it in large numbers of conidia—it yet appears of moment as it sustains the rather striking resemblance with respect to shape, curvature, and posture, which the conidia of *Stylopage rhynchospora* bear to the elongated secondary conidia known in several species of *Basidiobolus*.

COCHLONEMA PUMILUM Drechsler (**1939, 1945**)

In my earlier accounts of *Cochlonema pumilum* that fungus was set forth as forming a singe assimilative thallus in each infected specimen of the testaceous rhizopod *Euglypha levis* (Ehrenb.) Perty. A maize-meal-agar plate culture that had been planted with small quantities of leaf mold collected near Salisbury, Maryland, on November 16, 1951, showed 28 days later numerous individuals of the same host animal occupied by a single thallus (FIG. 10, *A*) and some few individuals containing 2 thalli of the parasite (FIG. 10, *B–D*). As the 2 thalli in each instance of double infection appeared of approximately equal size, it seems very probable that they developed from conidia ingested at nearly the same time. They always displayed parallel reproduction, each in most instances extending through the host orifice (FIG. 10, *B, C*) a

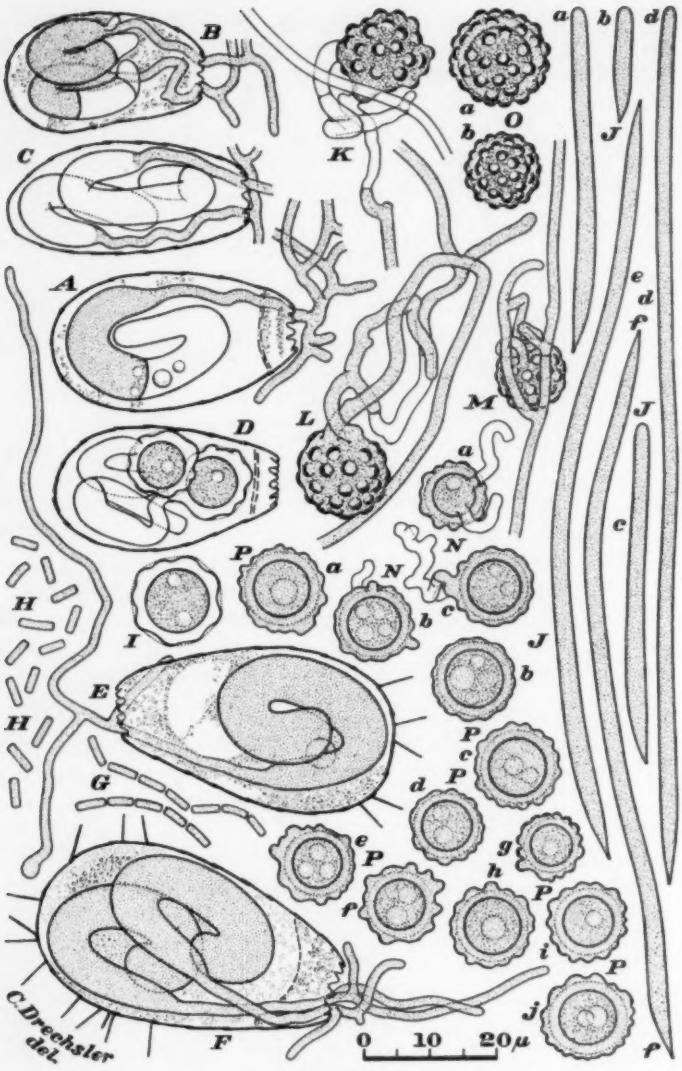

FIG. 10.

FIG. 10. Drawn at a uniform magnification with the aid of a camera lucida; × 1000. *A*, Test of *Euglypha levis* occupied by a thallus of *Cochlonema pumilum* that has become partly evacuated in extending asexual reproductive hyphae exter-

hypha terminating in conidial chains, though in some fewer instances each gave rise to a zygospore within the test of the host (FIG. 10, *D*).

Single and double infection of host animals by a fungus much resembling *Cochlonema pumilum*, but of somewhat larger dimensions, was observed in a maize-meal-agar plate culture that had been planted with a small quantity of leaf mold collected near Palatka, Florida, on January 1, 1954. The animal (FIG. 10, *E*, *F*) was a testaceous rhizopod similar in shape to the familiar *Euglypha levis*, but of larger size, often attaining a length of 50 μ and a width of 30 μ. It differed further in being sparsely beset over its posterior region with spines mostly 4 to 5 μ long. The coiled thallus, or assimilative hypha, found in the robust animal often measured 50 μ in length and 8 μ in greatest width near its broadly rounded distal end. Asexual reproduction resulted in a group of 3 to 5 ascending conidial chains mostly 100 to 300 μ long. On the moist substratum the chains readily became disjointed (FIG. 10, *G*), leaving the surface strewn with colorless cylindrical conidia 3.5 to 5.5 μ long and 1 to 1.4 μ wide (FIG. 10, *H*). When the fungus reproduced sexually, it formed within the animal's test 1 or 2 zygospores measuring sometimes as much as 14 μ in diameter and containing a spherical living cell about 9 μ in diameter (FIG. 10, *I*).

ZOOPAGE MITOSPORA Drechsler (1938)

For two decades after it was described *Zoopage mitospora* came under observation only occasionally and in relatively small quantity. Recently, however, it developed abundantly in more than a dozen maize-meal-agar plate cultures that had been planted with leaf mold collected in woods near Fort Collins, Colorado, and Steamboat Springs, Colorado, in July 1958. The filamentous conidia (FIG. 10, *J*, *a–f*) charac-

nally. *B*, *C*, Tests of *E. levis*, each occupied by 2 thalli of *C. pumilum* which have become partly emptied in extending asexual reproductive hyphae externally. *D*, Test of *E. levis* occupied by 2 thalli of *C. pumilum*, each with a mature zygospore attached to it. *E*, *F*, Two individuals of a spiny *Euglypha* occupied, respectively, by 1 and 2 thalli of a *Cochlonema* from Florida; each thallus is putting forth 1 or more conidiiferous hyphae. *G*, Disintegrating conidial chains of the *Cochlonema* from Florida. *H*, Detached conidia of the Florida *Cochlonema*. *I*, Mature zygospore of the Florida *Cochlonema* formed within test of spiny *Euglypha*. *J*, Detached conidia, *a–f*, of *Zoopage mitospora* (Colorado strain). *K–M*, Somewhat immature sexual reproductive units of *Z. mitospora* (Colorado strain). *N*, Mature zygospores, *a–c*, of *Z. mitospora* (Colorado strain) shown in optical section, with 1 or 2 hyphal parts. *O*, Mature zygospores, *a–b*, of *Z. mitospora* (Colorado strain), showing protuberances on their surface. *P*, Mature zygospores, *a–j*, of *Z. mitospora* (Colorado strain) in optical section, showing usual variations in size and shape.

teristic of the fungus became scattered over extensive areas in each culture, and in portions of these areas zygospores were also produced, though for the most part only rather scantity. Evidently the zygospores originated directly at the junction of two sexual branches, mostly 1.5 to 2.6 μ wide, which followed irregular courses and were often more elaborately intertwined than those in the 3 reproductive units shown in FIG. 10, *K–M*. On attaining definitive size the globose fusion cell became ruggedly ornamented with warty protuberances. Because of its prominent sculpture the mature yellowish zygospore (FIG. 10, *N*, *a–c*; *O*, *a*, *b*; *P*, *a–j*) did not clearly reveal the organization of its protoplasmic contents or the structure of its thick wall. It commonly varied in diameter from 9 to 14 μ, while the spherical protoplast contained within measured 5.7 to 8.7 μ in that dimension.

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FACTORS AFFECTING PARASITISM OF PIPTOCEPHALIS VIRGINIANA ON OTHER MUCORALES [1]

CHARLES R. BERRY [2]

(WITH 3 FIGURES)

After a century of scientific study of plant diseases, many of the basic problems of parasitism are still unsolved. The biochemistry of the nutritional relationships which are established between parasites and their hosts is poorly understood and the influence of environmental factors on parasitism is only partly understood. The most economical controls of fungus diseases of plants probably will not be achieved until a thorough understanding of host-parasite relationships is obtained.

A study of *Piptocephalis virginiana* Leadbeater and Mercer has given the answers to some questions concerning host-parasite relations. It is hoped that this information will open new avenues of approach for further study of the physiology of parasitism through the use of fungi parasitic on other fungi. This study has been one of considerable mycological interest because this species has been cultured only on other living Mucorales. One host, *Mycotypha microspora*, is considered by Wolf (**1957**) as a doubtful member of the Mucorales. However, the fact that this fungus is highly susceptible to parasitism by *P. virginiana* is considered as evidence that it belongs to the order of Mucorales. *P. xenophila* is the only known species of *Piptocephalis* that parasitizes fungi outside the Mucorales, namely certain Ascomycetes and Fungi Imperfecti (Dobbs and English, **1954**).

The results of a preliminary study of *Piptocephalis virginiana*, including host range, morphology, spore germination and a review of some of the pertinent literature, were presented in an earlier paper (Berry and Barnett, **1957**). A description of *P. virginiana* and a report of zygospore formation and synonymy of certain other species were given by

[1] This paper is based in part upon the Ph.D. dissertation of the author and is published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 602.

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Leadbeater and Mercer (**1956**, **1957a**, **1957b**). The work of Barnett and Lilly (**1958**) on the parasitism of *Calcarisporium parasiticum* Barnett (Barnett, **1958**) is of interest because many aspects of this study were similar to those encountered in the study of *P. virginiana*. The present paper reports the results of a study of the physiology of *P. virginiana* and factors affecting parasitism on some species of Mucorales.

MATERIALS AND METHODS

Since *Piptocephalis virginiana* has not been grown saprophytically, it was maintained on various ho. s. Spores of the parasite were obtained free of host spores by growing the parasite on *Choanephora cucurbitarum* in total darkness on a medium containing 20 g malt extract and 2 g yeast extract per liter. This technique, used to prevent sporulation of *C. cucurbitarum*, is similar to that reported earlier by Barnett and Lilly (**1950**, **1951**) and also described by Berry and Barnett (**1957**). The use of a mixed spore suspension often facilitated the inoculation of host and parasite together at the same point. A mixed spore suspension was prepared by growing the parasite and desired host together in test tubes on agar slants. By adding sterile distilled water to the tube cultures, mixed inoculum suitable for many experiments was easily obtained.

The temperature range for growth of *P. virginiana* was determined by placing agar plates inoculated with mixed spore suspensions of a host fungus and the parasite in constant-temperature rooms, refrigerators and incubators at temperatures of every 5° from 5° to 35° C. The medium used contained 3 g malt extract, 1 g yeast extract and 20 g agar per liter, at an initial pH of 6.0.

An extensive study was made to determine the effect of host nutrition on growth of the parasite. For this study, the following basal semi-synthetic medium recommended by Lilly and Barnett (**1951**) was used:

Carbon source	3.0 g
Nitrogen source yielding	0.2 g nitrogen
KH_2PO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
Micro element solution[3]	2.0 ml
Thiamine	100.0 μg
Agar	20.0 g
Water (double distilled)	1.0 l
pH (initial)	6.0

A total of 40 media varying in carbon and nitrogen sources were used. Each of ten different nitrogen sources was combined in one series

[3] This amount of solution contained: Fe^{+++}, 0.2 mg; Zn^{++}, 0.2 mg; Mn^{++}, 0.1 mg; Ca^{++}, 10 mg.

with glucose, which is almost universally used by fungi, and in another series with L-sorbose, which is poorly utilized by most fungi. In a similar way, asparagine, as a good nitrogen source, and phenylalanine, as a poor nitrogen source, were combined in media with ten carbon sources.

Ten host fungi were selected and grown with the parasite on each medium given above. Two plates of each host-medium combination were inoculated with a mixed spore suspension in three places by means of a wire loop, or a drop from a pipette. This provided a total of six replications per experiment. The plates were incubated at 25° and were examined after five to seven days and again after ten to fourteen days. Each experiment was repeated at least once. Growth of both host and parasite was recorded. Host growth was rated poor, medium, or good. Parasite growth was rated 0, 1, 2, 3, or 4.

EXPERIMENTAL RESULTS

Spore germination. Most of the results of the spore-germination studies have already been reported (Berry and Barnett, **1957**). In that paper it was noted that the only known synthetic media capable of supporting germination of *Piptocephalis virginiana* spores contained glutamic acid. Further experiments have shown that this is true only when glutamic acid is present during autoclaving. When glutamic acid was withheld from the medium, autoclaved separately, and then added, the medium did not support germination of *P. virginiana* spores.

Temperature range for growth of the parasite. The hosts selected for this study and their approximate temperature ranges were as follows: *Thamnidium elegans,* 5°–25°; *Helicostylum* sp., 10°–30°; *Mortierella pusilla,* 10°–30°; and *Choanephora cucurbitarum,* 10°–35°. Growth of the parasite occurred on all hosts at 25° after 3 days and after several days the approximate favorable temperature range was 15°–25°.

Two of these hosts showed greater susceptibility at certain temperatures than at others. *T. elegans* was highly susceptible at 25°, but showed resistance at 15° and 20°. *Helicostylum* sp., on the other hand, was susceptible at 15° and 20°, but resistant at 25°.

Effect of the parasite on host growth. Piptocephalis virginiana was able to grow well without causing any apparent harm to the host in most combinations. However, the parasite did reduce growth of *Mycotypha microspora* on a glucose-glutamic acid medium (FIG. 1). This was shown by comparing dry mycelial weights of the host when grown alone in a liquid medium with those of the host and parasite cultured together. After 17 days incubation at 25°, dry mycelium of the host weighed

more than 25 mg while the combined weight of host and parasite was less than 5 mg.

The effect of age of mycelium on parasitism. Parasitism of *Piptocephalis virginiana* on *Helicostylum* sp. was affected by the maturity of the host-cell wall. When an agar plate was inoculated in the center with spores of both the parasite and host a trace of parasitism occurred at the point of inoculation, but the parasite did not spread. When agar plates were flooded with parasite spores and inoculated in the center only with the host, the parasite grew as a ring at the outer edges of the host colony where the host hyphae were young and immature and the

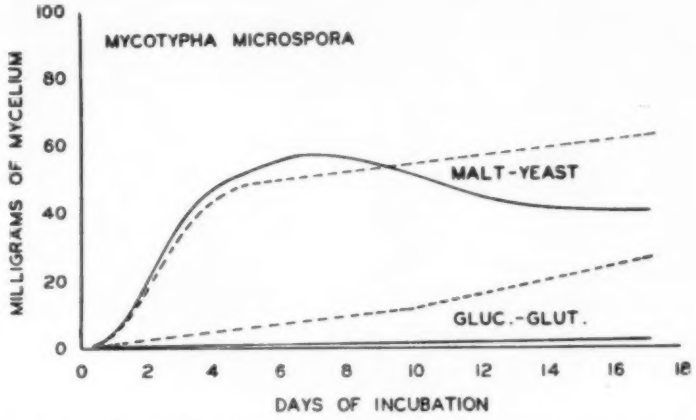

FIG. 1. Effect of the parasite on growth of *Mycotypha microspora* on malt-extract-yeast-extract and glucose-glutamic acid media. Broken lines indicate the host when grown alone and solid lines indicate the host when parasitized.

zone occupied by the parasite enlarged as the host colony enlarged. This strongly suggests that the mature wall of the older hyphae of *Helicostylum* sp. is resistant to penetration by the parasite. It also suggests that continued multiple penetration is needed for survival of the parasite, since the parasite ceased to grow in the areas where the host hyphae had matured.

The effect of host nutrition on growth of the parasite. The growth and development of an obligate parasite means that a compatible nutritional relationship has been established between the parasite and its host. Since the metabolism of an organism is influenced by its nutrition, the medium on which the host fungus is grown could indirectly have a marked effect on the parasite. This was borne out in an experiment in

which the carbon and nitrogen sources were varied in the host media. The results obtained on a series of media containing glucose and varied sources of nitrogen are presented in TABLE I. An example of how changing the nitrogen source sometimes affects the degree of parasitism is illustrated in FIG. 2.

A similar experiment was conducted in which the same carbon and nitrogen sources were used throughout, but their concentrations were varied. One series of media contained 3 g of glucose per liter, while

TABLE I

GROWTH OF PIPTOCEPHALIS VIRGINIANA ON 10 HOSTS GROWN ON MEDIA CONTAINING GLUCOSE IN COMBINATION WITH 10 NITROGEN SOURCES[1]

Ten host fungi	Alanine	Asparagine	Ammonium sulfate	Ammonium tartrate	Casein hydrolysate	Glutamic acid	Glycine	Phenylalanine	Potassium nitrate	Urea
Absidia orchidis	2	2	p1	p2	4	3	2	1	p1	3
Helicostylum sp.	p0	p0	—	p0	0	p1	p0	p0	—	p0
Mortierella pusilla	2	2	0	0	3	3	3	p2	p0	2
Mucor ramannianus	2	2	1	1	2	2	1	0	p0	2
Mucor spinescens	2	2	0	2	2	2	2	0	1	0
Mycotypha microspora	—	p2	p3	p3	3	p2	p2	—	p1	p2
Rhizopus nigricans	1	2	0	2	1	1	2	2	p1	0
Syncephalastrum racemosum	2	2	1	1	2	2	2	1	2	1
Thamnidium elegans	2	2	2	2	3	2	2	2	p1	2
Zygorhynchus vuilleminii	2	1	1	2	2	2	1	2	p1	1

[1] Estimated growth of the parasite is rated from 0 to 4. The letter "p" indicates poor growth of host, and the dash line indicates no growth. In all other cases the hosts made moderate to good growth. Cultures were 5 to 7 days old.

glutamic acid was varied from 0.25 to 8.0 g. In the second series glutamic acid was constant at 2.0 g and glucose was varied from 0.75 to 24 g. Parasitism was tested on ten different hosts grown on all the above variations of the glucose-glutamic acid medium. The degree of parasitism on some of these hosts was approximately the same, as long as the ratio between the amount of carbon and nitrogen present in the media was the same. On *Mucor ramannianus*, the growth of the parasite was approximately the same on each medium that contained 3 g of glucose for every 1 g of glutamic acid. With other hosts, the degree of parasitism changed when the concentrations of carbon and nitrogen changed, even though the carbon-nitrogen ratio remained the same. For example, when the medium contained 6 g of glucose and 2 g of glutamic acid the degree of parasitism on *Mortierella pusilla* was 2.

When the medium contained 24 g of glucose and 8 g of glutamic acid, still a ratio of 3:1, there was no growth of the parasite (FIG. 3). This appears to be due largely to the increased amount of glucose, since parasitism on media containing 3 g of glucose and 8 g of glutamic acid was rated 3. There was a trace of parasitism, however, on a medium containing 24 g of glucose and 2 g of glutamic acid. The greatest change in the degree of parasitism brought about by changing the concentrations of components of the medium occurred on *Mucor ramannianus*. On this host when the amount of glucose was kept constant at 3 g per liter,

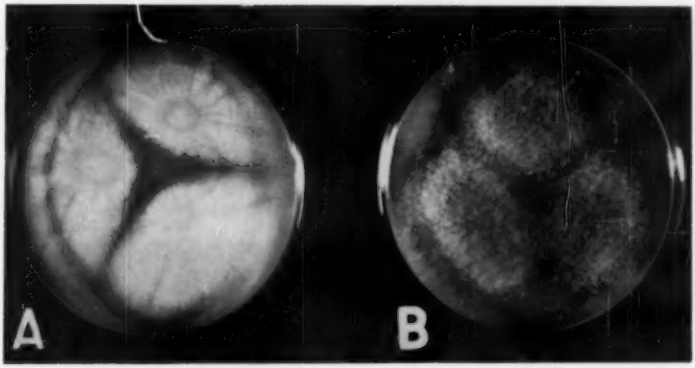

FIG. 2. Comparison of parasitism on *Mortierella pusilla* grown on media containing: (A), glucose and ammonium sulfate (no parasitism) and (B), glucose and glutamic acid (heavy parasitism).

and the amount of glutamic acid per liter was varied, the parasitism ratings were as follows: 0.25 g, 0; 0.5 g, 1; 1.0 g, 2; 2 g, 2; 4 g, 3; 8 g, 3. Host growth was rated moderate in each case.

DISCUSSION

Studies of the physiology of obligate parasites are complicated by the necessity of growing the parasites on their hosts. Repeated attempts were made to grow *Piptocephalis virginiana* in the absence of a host, but none was successful. There was no growth of the parasite in close proximity to a host when the two were separated by cellophane or sintered-glass filters. All evidence obtained thus far indicates that *P. virginiana* obtains its nutrients only from living mycelium of a susceptible host. Because of the obligate nature of the parasite, it was not possible to measure accurately the growth of the parasite. Neither was it possible to measure host growth alone when the host was supporting

the parasite. For these reasons it was necessary to estimate growth of the host and parasite in much of the work. It is believed, however, that since the variations in growth of the various hosts and the parasite were so great, the method was satisfactory for this purpose.

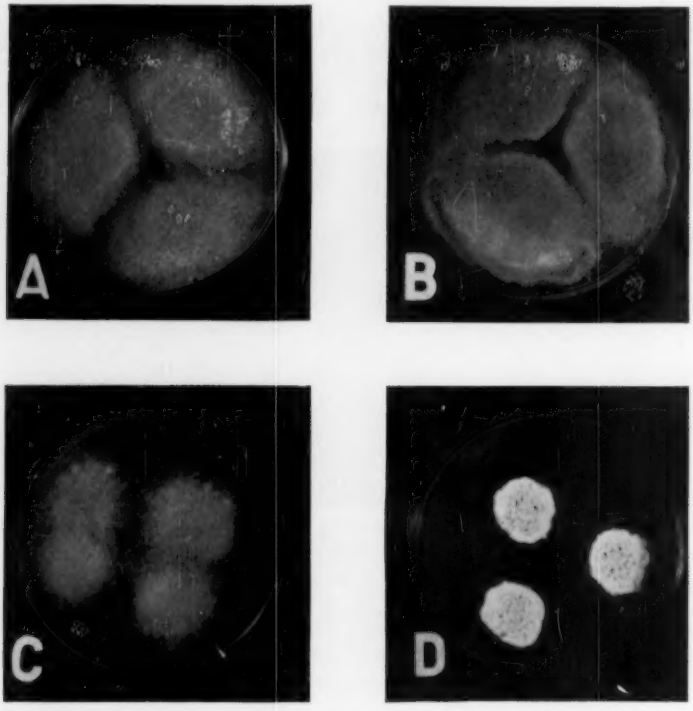

FIG. 3. Comparison of parasitism on *Mucor ramannianus* and *Mortierella pusilla* on glucose-glutamic-acid media of different concentrations. Parasitism on *M. ramannianus* (above) was approximately the same when the medium contained: (A), 6 g glucose and 2 g glutamic acid, or (B), 24 g glucose and 8 g glutamic acid. Parasitism on *M. pusilla* (below) was good when the medium contained: (C), 6 g glucose and 2 g of glutamic acid, but did not occur when the medium contained (D), 24 g glucose and 8 g glutamic acid.

In one experiment it was possible to determine the inhibitory effect of the parasite on host growth in liquid media by obtaining the dry weight of the mycelium. When *Mycotypha microspora* was cultured on a glucose-glutamic acid medium, the dry mycelium of the host when grown alone was five times as heavy as the mycelium of the host and

parasite combined. Since some of the weight of the combination was due to parasite mycelium, the host actually made less than one-fifth as much growth when supporting the parasite as it did when growing alone.

It was established that glutamic acid itself would not support germination of the parasite spores even though the only synthetic media which allowed germination contained glutamic acid. It appears that the spore-germination factor is formed by a reaction between glutamic acid and glucose or some other simple sugar during the process of autoclaving.

The temperature range in which the parasite grew was not the same with all hosts tested. Differences in susceptibility were best illustrated by *Helicostylum* sp. and *Thamnidium elegans*. *Helicostylum* sp. was highly susceptible at 15° and 20°, and resistant at 25°; while *T. elegans* was resistant at 15° and 20°, but susceptible at 25°.

The maturity of the host cell wall was shown to be an important factor in resistance in the case of *Helicostylum* sp., which was parasitized only where the hyphae were immature.

In the nutritional study, the parasite was cultivated on ten hosts grown on various media. The degree of parasitism on any host could be altered by changing the medium. It was not uncommon for the parasite to fail to grow on a given host on one medium and to make moderate to excellent growth on the same host growing on a different medium. It is concluded that a change in the internal constituents of the host mycelium probably accompanies a change in the medium.

SUMMARY

Spores of *Piptocephalis virginiana* germinated on synthetic media which contained glutamic acid, but only when glutamic acid was autoclaved with the other components of the medium. When glutamic acid was withheld from the medium, autoclaved separately, and then added, the medium did not support germination.

P. virginiana parasitized *Choanephora cucurbitarum*, *Thamnidium elegans*, *Mortierella pusilla* and *Helicostylum* sp. at 20° and 25°. In addition, *M. pusilla* and *Helicostylum* sp. were parasitized at 15°. Susceptibility of two of these hosts was strongly affected by temperature. *T. elegans* was highly susceptible at 25°, but resistant at 15° and 20°. *Helicostylum* sp. was susceptible at 15° and 20°, but resistant at 25°. All of these host fungi grew moderate to well at temperatures from 15° to 30° and poorly at 10°.

Parasitism of *Mycotypha microspora* by *P. virginiana* reduced host growth on a glucose-glutamic acid medium. After 17 days at 25°, the host growing alone produced more than 25 mg of dry mycelium. The

weight of the combined dry mycelium of host and parasite was only 5 mg. Such inhibition was not evident on other media or with other hosts.

P. virginiana readily attacked young hyphae of *Helicostylum* sp., but did not attack old, mature hyphae of that fungus.

Changes in nutrition of the host were found to influence greatly the growth of the parasite, even when growth of the host was only slightly, or not at all, affected. In this work, carbon and nitrogen sources were varied and several different host fungi were tested. When concentrations and proportions of glutamic acid and glucose were varied, growth of the parasite often varied as much as when the carbon or nitrogen sources differed.

ACKNOWLEDGMENTS

This work was financed partially by a grant from the National Science Foundation. The author expresses his appreciation to Dr. H. L. Barnett who directed the work.

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THE HELOTIALES OF THE MUSSOORIE HILLS — II

K. S. Thind, Edith K. Cash, and Pritam Singh

(with 13 figures)

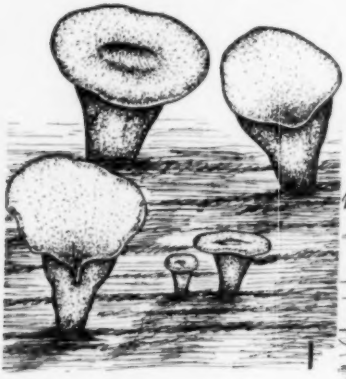
This paper records additional species of the Helotiales from the Mussoorie Hills (5000–7500 ft altitude in the North Western Himalayas) as a part of the study of the fungus flora of that region undertaken by the senior author and his students. The first contribution (**2**) described eight known species. This second contribution deals with three new species belonging to *Midotis*, *Helotium* and *Rutstroemia*. The fruit bodies are described from fresh material as well as from that preserved in alcohol-formalin. The numbers of the species are the serial numbers of the senior author's helotioid flora of the Mussoorie Hills. Type collections have been deposited in the Herbarium of the Panjab University and duplicate material is in the U. S. National Fungus Collections, Beltsville, Maryland.

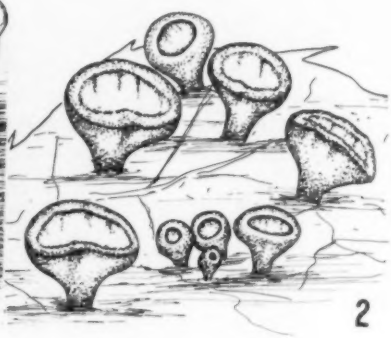
9. **Midotis indica**, sp. nov. Figs. 1, 4, 7, 10

Apothecia 3–15 mm in diam., stipitata, gregaria usque dense conferta, interdum regularia sed saepius contorta et in maturitate inaequilateraliter elongata, umbilicata vel spathulata, griseo-brunnea, carnosa usque lenta, pelliculosa, margine incurvato, hymenio pallide griseo-brunneo, sicco obscuriori usque paene atro, glabro, centro depresso; stipes brevis, cylindricus, supra in apothecium dilatans, conspicue rimosus costatusque, 2–5.5 × 1.5–3 mm; asci cylindrico-elongati, ad apices rotundati, in pedicellum longum attenuati, ope iodi non caerulescentes, inoperculati, 92–123 × 4.5–6.3 μ; ascosporae 8, uniseriatae, paralleles usque oblique dispositae, hyalinae, ellipsoideae, rectae, glabrae, demum uniseptatae, ad apices subacutae 7.5–9.7 × 2.2–3.7 μ; paraphyses eramosae, 95–132 × 1–2.7 μ, filiformes vel ad apices usque 3.6 μ inflatae; stratum medianum excipuli plectenchymaticum, ex hyphis intertextis tenuibus pallide brunneis compositum.

Apothecia 3–15 mm in diameter, stipitate, gregarious, sometimes densely crowded, regular or contorted, often elongated on one side and thus appearing laterally stipitate, umbilicate to spathulate, dark grayish brown (dark brown with an ash-colored shade), fleshy or very slightly tough, furfuraceous; external surface dark brown, turning black on drying, pelliculose from minute, imbricated, flattened squamules or scales composed of clumps of subglobose to angular, dark-walled cells; hy-

menium dark grayish brown (olivaceous black to fuscous-black of Ridgway) lighter than the external surface, turning darker brown to almost black on drying, smooth, plane but depressed in the center; stipe

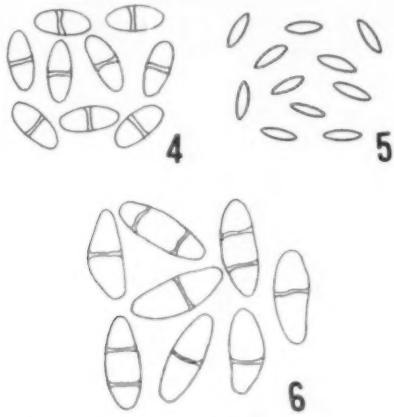




FIGS. 1–3. Apothecia. FIG. 1. *Midotis indica*, × 1.5. FIG. 2. *Helotium quercifolium*, × 35. FIG. 3. *Rutstroemia sepiacea*, × 5.

short, cylindric, expanding above into the apothecium, pelliculose and dark brown like the exterior of the apothecium, conspicuously marked by grooves and ridges which extend above into the apothecium, 2–5.5 × 1.5–3 mm; *asci* cylindric-elongate, rounded at the apices, narrowed

below into a long stem-like base, not turning blue with iodine, inoperculate, 92–123 × 4.5–6.3 μ; *ascospores* 8, uniseriate, parallel to oblique with ends overlapping, hyaline, ellipsoid, straight, smooth, rounded at the ends, non-septate at first, then uniseptate, eguttulate, 7.5–9.7 × 2.2–3.7 μ; *paraphyses* filiform, simple, non-septate, the apices subacute and filled with homogeneous contents, slender, 95–132 × 1–2.7 μ or sometimes enlarged to 3.2–3.6 μ at the tips; hypothecium pale brownish; medullary layer pale brown, plectenchymatic, composed of very fine subhyaline to yellowish brown hyphae; cortex pseudoparenchymatic, of dark brown, thick-walled cells, often aggregated in clumps or clusters.



FIGS. 4–6. Ascospores. FIG. 4. *Midotis indica*, × 950. FIG. 5. *Helotium quercifolium*, × 1150. FIG. 6. *Rutstroemia sepiacea*, × 950.

Collected on dead and decaying stems of oak: India (The Park, Mussoorie, August 16, 1956, No. *319*, TYPE).

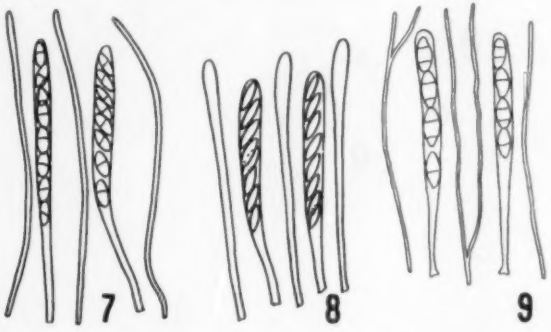
This fungus would appear to belong to *Midotis* in having dark apothecia which are vertically elongated on one side and in the presence of a medullary layer of subhyaline to brown hyphae in the exciple. It is characterized by dark, stipitate, umbilicate apothecia, pelliculose external surface and uniseptate, hyaline, ellipsoid ascospores. The paraphyses are extremely variable in width; some are very fine and filiform (1–1.8 μ wide) and not enlarged at their apices, while the majority are much wider, enlarged at the tips (1.8–2.7 μ wide, 3.2–3.6 μ at the tip) and slightly thick-walled.

In Seaver's key (1) this Mussoorie fungus comes very near to *M. occidentalis* Durand, a species occurring in Jamaica, but differs from

the latter in the larger size of apothecia, spores, asci and paraphyses. The paraphyses are also enlarged at the tip and the spores uniseptate. The Indian fungus does not seem to belong to any of the reported species of *Midotis*.

10. **Helotium quercifolium** sp. nov. FIGS. 2, 5, 8, 11

Apothecia 0.3–0.7 mm in diam., 0.3–0.6 mm alta, gregaria, primum globosa dein cupulata, molle carnosa, pruinosa, flavida, sicca ochraceo-lutea, hymenio ochraceo-aurantio; stipes brevis, pruinosus, 140–210 × 70–98 μ; asci clavati, ad apices rotundati, in basim brevem attenuati, ope iodi non caerulescentes, inoperculati, 26–33 × 2.2–3.7 μ; ascosporae 8, uniseriatae, hyalinae, ellipsoideae vel fusoideae, apicibus attenuatis, eseptatae, glabrae. 3.7–6 × 1.5–2.2 μ, paraphyses clavatae, simplices, hyalinae, 31–42.7 × 0.8–1.8 μ, ad apices usque ad 1.5–3 μ inflatae.



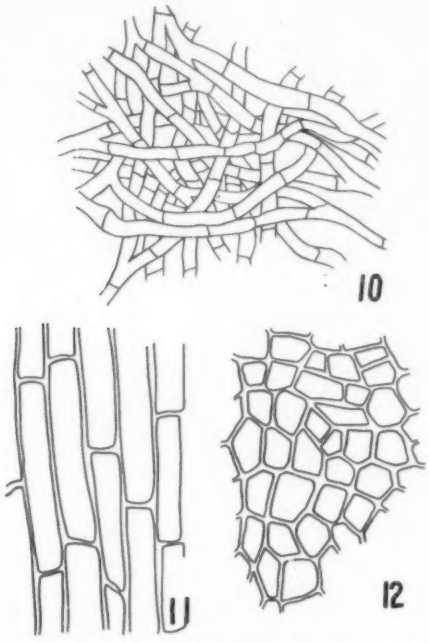
FIGS. 7–9. Asci and paraphyses. FIG. 7. *Midotis indica*, × 400. FIG. 8. *Helotium quercifolium*, × 1150. FIG. 9. *Rutstroemia sepiacea*, × 400.

Apothecia 0.3–0.7 mm in diameter and 0.3–0.6 mm in height, loosely to densely gregarious, globose and closed when young, expanding to become shallow cupulate, regular, light to deeper yellow, soft, fleshy, pruinose, stipitate, on drying usually irregular in shape, deeper, and brighter in color; external surface light or deeper yellow (ochraceous buff to tilleul buff of Ridgway), pruinose; excipular cells subhyaline individually, pale yellow in mass, rectangular, slightly thick-walled, 3–6.5 μ wide; margin entire, thickened and translucent; hymenium light to deeper yellow (ochraceous orange of Ridgway), darker than the external surface, concave, smooth; stipe short, cylindrical, expanding above into the apothecium, concolorous with the external surface, pruinose, 140–210 × 70–98 μ; *asci* clavate, with apex rounded, tapering below into a short stem-like base, not turning blue with iodine, inoperculate, 26–33 × 2.2–3.7 μ; *ascospores* 8, uniseriate, oblique with ends overlapping, hyaline, ellipsoid or fusoid, ends narrowed, non-septate, smooth, eguttu-

late, 3.7–6 × 1.5–2.2 μ; *paraphyses* clavate, non-septate, simple, hyaline, 31–42.7 × 0.8–1.8 μ, enlarged to 1.5–3 μ at the tips.

Collected on dead leaves of *Quercus incana* Roxb. in oak forest: India (The Spring Road, Mussoorie, September 8, 1956, No. *320*, TYPE).

This beautiful fungus apparently comes close to *Helotium sparsum* Boud. (*H. naviculasporum* Ell.) and *Pezizella jaapii* Rehm, which also



FIGS. 10–12. Excipulum. FIG. 10. *Midotis indica*, × 400. FIG. 11. *Helotium quercifolium*, × 1150. FIG. 12. *Rutstroemia sepiacea*, × 400.

occur on oak leaves, but differs in its much smaller asci, spores and paraphyses. In Seaver's key it comes quite near to *H. midlandense* W. L. White, from which it also differs in smaller dimensions and non-septate paraphyses. Thus this fungus appears to be unreported.

11. **Rutstroemia sepiacea** sp. nov. FIGS. 3, 6, 9, 12, 13

Apothecia stipitata, 2.5–7 mm in diam. in areis atratis petiolorum gregaria vel dispersa, interdum dense conferta, disciformia, lenta, sepiacea, hymenio fusco-brunneo, plano-convexo; stipes cylindricus, fulvus, infra obscurior, furfuraceus;

asci cylindrici, ad apices rotundati, bases versus gradatim attenuati, ope iodi non caerulescentes, inoperculati, 72–83 × 5.4–7.2 μ; ascosporae 4, uniseriatae, paralleles, subhyalinae vel pallide ochraceae, glabrae, ellipsoideae vel subovoideae, 1–2-pseudoseptatae, 11.2–14.2 × 3.7–5.3 μ; paraphyses filiformes, simplices vel ramosae, pallide flavidulae, eseptatae, 81–108 × 1–1.8 μ; hypothecium tenue, stratum medianum crassum, subhyalinum, plectenchymaticum, ex hyphis tenuibus 1.5–2 μ diam. flavidulis compositum; cortex subhyalinus, prosenchymaticus, hyphis fulvobrunneis, verrucosis, dense fasciculatis, 2.7–3.6 μ crassis, strigosus.

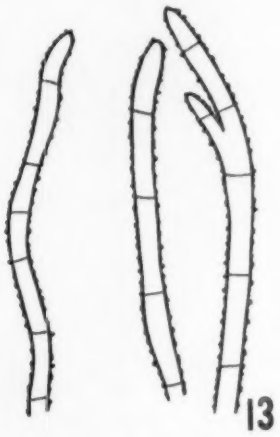


FIG. 13. Excipular hyhpae of *Rutstroemia sepiacea*, × 400.

Apothecia stipitate, 2.5–7 mm in diameter, on blackened areas of petioles, gregarious to densely crowded or sometimes scattered, discoid, slightly tough, externally slightly darker than the hymenium (warm sepia to bone brown of Ridgway), delicately furfuraceous, excipular cells rectangular, slightly thick-walled, individually subhyaline, light yellowish brown to yellowish brown in mass, 5.4–25 μ wide; margin entire, appearing dentate from clusters of hyphae, slightly elevated; hymenium yellowish brown, even, smooth, paler than the external surface, plane to convex; stipe cylindrical, expanding above into the apothecium, yellowish brown, darker than the exterior of the apothecium, blackish below, furfuraceous, 0.2–1.2 cm long, 350–770 μ wide; hypothecium thin; ectal layer subhyaline, prosenchymatic, covered by yellow-brown, verrucose, fasciculate hyphae; hyphae on the stipe and external surface of the apothecium mostly simple, sometimes branched, yellowish brown to deep brown, septate, flexuous, distinctly roughened, usually rounded at the tips, closely aggregated into dark brown to blackish, patch-like, discrete clusters along the margin, giving it a dentate appearance, 35–215

× 2.6–3.6 μ; inner layer of the exciple thick, subhyaline, plectenchymatic, composed of very thin, smooth, subhyaline to yellowish hyphae 1.5–2 μ in diameter; *asci* cylindrical, rounded at the apices, tapering gradually below into a stem-like base, not turning blue with iodine, inoperculate, 72–83 × 5.4–7.2 μ; *ascospores* 4, uniseriate, parallel with ends overlapping or not, subhyaline to ochraceous, ellipsoid to subovoid, smooth, straight or slightly curved, often slightly narrowed at the lower end, pseudoseptate, 11.2–14.2 × 3.7–5.3 μ; *paraphyses* filiform, simple or branched, very pale yellow or ochraceous, non-septate, not enlarged at the apices, 81–108 × 1–1.8 μ.

Collected on fallen petioles in oak forest: India (The Park, Mussoorie, August 28, 1956, No. *321,* TYPE).

This very abundant Mussoorie fungus evidently belongs to the genus *Rutstroemia* sensu W. L. White nec Karst. but does not fit in any of the species hitherto reported. Although it closely resembles *R. petiolorum* (Rob.) W. L. White (*Calycina petiolorum* (Rob.) Seaver) and *R. sydowiana* (Rehm) W. L. White, both of which occur on petioles of oak leaves, the Indian fungus does not agree with either of these species. It differs from *R. petiolorum* in having smaller asci and spores and paraphyses not enlarged at the tips. The external surface, moreover, shows no longitudinal striations and the ascospores are pseudoseptate. *R. boliviana* Cash on leaf petioles from Bolivia and *R. corneri* Cash collected in Brazil on dead leaves are both distinct from *R. sepiacea* in the tawny-yellow color of the apothecia, which turn reddish brown when dried.

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NEW OR NOTEWORTHY TREMELLALES FROM BOLIVIA

BERNARD LOWY [1]

(WITH 4 FIGURES)

Early in 1958 I received for identification a number of tremellaceous fungi that had been collected by Dr. Rolf Singer in Bolivia. Later, in November and December of the same year I visited Bolivia in order to gather further information on the Tremellales. This paper summarizes the results of the investigation.

When Patouillard described *Platygloea javanica* in 1898 only five other species were known. Recently, Bandoni (**1**) included 23 species in the genus, of which *P. javanica* was still the only foliose member, the others being resupinate and mostly inconspicuous. Apart from other fundamental differences among the species which make a reconsideration of their status desirable, the finding of a second foliose species emphasizes the need for a revision of the genus. The new fungus to be described is easily placed near *P. javanica* yet differs from it significantly in external and internal characteristics. Through the kindness of Dr. I. M. Lamb I have been able to examine the type of *P. javanica*, in its present condition, a blackish, lobed fragment about 6 × 4 mm. It conforms for the most part with Patouillard's description but I found the basidia somewhat larger than he indicated and frequently 3–5 septate. The hyphae bore numerous clamp connections. No spores were found. It is clear from a study of *P. javanica* and the new species that they should no longer be maintained within an already heterogeneous group. It is therefore proposed that a new genus be established to accommodate these and other foliose members that might be found in the future.

PHYLLOGLOEA gen. nov.

Fructificatio gelatinosa, foliacea vel lobata; hymenium bilaterale; basidia cylindracea vel subclavata, aseptata vel transverse septata.

Type species: *Phyllogloea Singeri.*

Fructification foliose, erect to lobate, gelatinous, drying horny and convoluted; hymenium bilateral; basidia cylindrical to subclavate, aseptate to transversely septate.

[1] The writer extends his grateful acknowledgment to the Fulbright Commission and to Louisiana State University for their support of this investigation.

Phyllogloea Singeri sp. nov. FIGS. 3, 4

Fructificatio foliacea, firme gelatinosa, aurantio-rubra ("carrot-red," Maerz & Paul), 20 mm in alt.; sicca cornea, aurantio-lutea; hyphae nodosae; basidia 3–4 septata, (35–)45–68(–76) × 5–6 μ; basidiosporae ovoideae, hyalinae, 9.5–12 × 5–6 μ, per promycelium germinantes.

TYPE: *Singer B1329*, 22–II–1956, Prov. Nor-Yungas, Dpto. La Paz. On stem of *Capellaria* sp. The species is named for Dr. Rolf Singer.

Fructification foliose, firm-gelatinous, "carrot-red" (Maerz & Paul) when fresh, becoming horny when dry, fading to light yellowish-orange; 20 mm in greatest dimension; hymenium bilateral; hyphae with clamp connections; basidia 3–4 septate, (35–)45–68(–76) × 5–6 μ; basidiospores ovoid, hyaline, 9.5–12 × 5–6 μ; germinating by repetition.

The following new combination is proposed.

Phyllogloea javanica (Pat.) comb. nov.

Platygloea javanica Pat., Bull. Soc. Myc. Fr. **14**: 190. 1898.

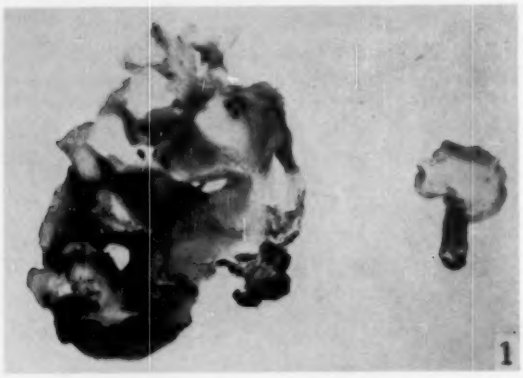
The remaining species of *Platygloea* need to be revised, possibly along the lines suggested by Olive (**6**). His redefinition of the genus does not take into account the foliose species, and since these are now removed from *Platygloea* his description may still serve as a basis for evaluating the resupinate forms, including a new species recently reported from South Africa by Talbot (**9**).

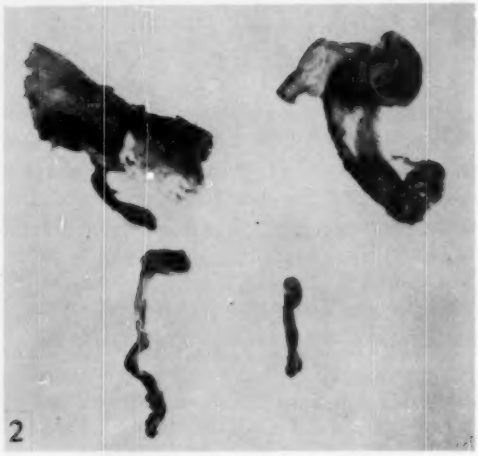
AURICULARIA DELICATA (Fr.) Henn., Jahrb. Wiss. Bot. **17**: 493. 1893.

Fructifications up to 3.5 cm broad, cupulate, sessile, tough-gelatinous, tan to light brown; hymenium inferior, strongly reticulate; abhymenium finely tomentose; drying brownish, horny; basidia cylindrical, becoming transversely 3-septate, 42–50 × 4–5.5 μ; basidiospores curved-cylindrical, 9.5–12 × 5–6 μ.

Singer B1249, 17–II–1956, Prov. Nor-Yungas, Dpto. La Paz; *Lowy B103*, Siberia, 3–XII–1958, Prov. Valle Grande, Dpto. Cochabamba.

This is known mostly from subtropical and tropical regions of the world and is one of the most easily identified members of the genus, with its strongly reticulate hymenium. Native Bolivian informants told me that this species and *A. fuscosuccinea* were used as food and locally called "orejas" or "orejitas" (ears). It is prepared by boiling in water, then sliced and eaten with salads. I found that a similar use was made of these fungi (including *A. polytricha*) by native populations along the Amazon in the region of Iquitos, Perú, as well as in the vicinity of Pucallpa on the Ucayali river.





FIGS. 1-3.

FIG. 1. *Dacryopinax Indacocheae* (TYPE), × 2. FIG. 2. *Dacryopinax yungensis* (TYPE), × 2. FIG. 3. *Phyllogloea Singeri* (TYPE), × 2.

AURICULARIA FUSCOSUCCINEA (Mont.) Farl., Bibl. Index **1**: 307. 1905.

Fructifications up to 6 cm broad, rubbery-gelatinous, cupulate to auriform, reddish-brown to rosy-red, sessile to substipitate; hymenium inferior, smooth to weakly and irregularly veined but never reticulate; well-defined central medulla seen in transverse section; abhymenium tomentose; drying horny with reddish or red-brown hues; basidia cylindrical, transversely 3-septate, 55–65 × 4–6 μ; basidiospores allantoid to curved-cylindrical, 11–13 × 4–4.5 μ.

Singer B993, 8–II–1956, Coroico, Prov. Nor-Yungas, Dpto. La Paz; *Singer B1680,* Guayaramerin, 5–III–1956, Prov. Vaca Diez, Dpto. Beni; *Lowy B96,* Siberia, 1–XII–1958, Prov. Valle Grande, Dpto. Cochabamba.

When this is found in the living condition in the field its rosy to pinkish color, unique among auriculariias, is sufficient to identify it. On drying, the color fades rapidly and is useless as a diagnostic characteristic.

AURICULARIA POLYTRICHA (Mont.) Sacc., Atti R. Inst. Veneto VI **3**: 722. 1855.

Fructifications up to 5 cm broad, rubbery-gelatinous, generally substipitate, often strongly cupulate, drying brownish to almost black; hymenium inferior, predominantly smooth; abhymenium densely pilose, appearing greyish when dry; basidia cylindrical, 50–60 × 4–5 μ, becoming 3-septate; basidiospores curved-cylindrical, 12–15 × 5–6 μ.

Lowy B119, Siberia, 2–XII–1958, Prov. Valle Grande, Dpto. Cochabamba; *Rusby 29,* 15–VII–1921; *Rusby 2118,* 1921 (BPI).

To anyone who has found this fungus in the field, the densely pilose abhymenium, strongly cupulate form and dark, smooth hymenium are usually sufficient for identification. In doubtful cases, especially when dealing with herbarium material, I find that the internal hyphal zonation as seen in free-hand sections is diagnostic. Details of this method are explained elsewhere (**3**).

In a recent paper by Olive (**7**), the author states that by the use of the zonation method he cannot separate *A. mesenterica* from *A. auricula* or *A. ornata* and reduces these names to synonymy. He also doubts that *A. auricula* and *A. polytricha* are distinct and further, in his opinion, *A. polytricha* and *A. cornea* are indistinguishable. Finally, he concludes that "the system is inadequate for accurate identification of species." This generalization is made in spite of the fact that only part of the evidence is examined. Fifty per cent of the species that I include

in the genus are not discussed. Concerning *A. mesenterica*, Olive says: "I have examined collections in the New York Botanical Garden identified as *A. ornata* and *A. mesenterica* and find no consistent differences among them." I do not know the number of specimens upon which this

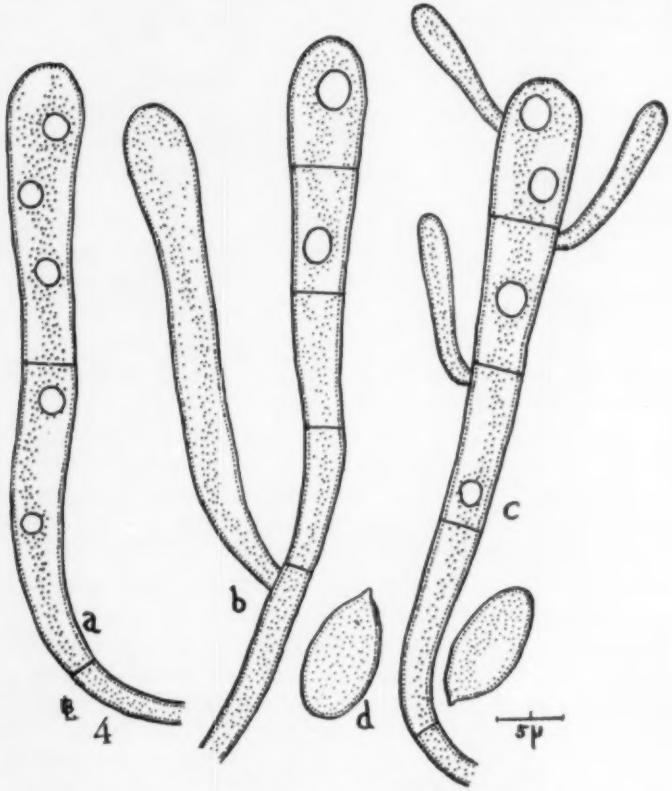


FIG. 4. *Phyllogloea Singeri* (TYPE). a–b. development of basidia; c. basidium with developing epibasidia; d. basidiospore.

conclusion rests but it seems relevant to indicate that after examining more than 200 collections of *A. mesenterica* from 42 countries and of *A. ornata* and *A. peltata* from six Pacific Islands and other localities, I find sufficient evidence to justify their separation as valid species. Regarding *A. peltata*, the fructifications are macroscopically distinct

enough to be separable from *A. ornata* and *A. mesenterica*. Photographs published by the writer (**4**, p. 672, figs. A, B, C, *A. mesenterica, A. ornata;* p. 674, fig. B, *A. peltata*) show this. When Olive reported finding the hairs of *A. peltata* 780 μ long, rather than 70–80 μ as I indicate, he was obviously measuring the mycelial fringe plainly visible in the photograph referred to. As in other species of *Auricularia* the characteristic hairs are abhymenial not marginal.

The confusion between *A. auricula* and *A. polytricha* in the minds of some authors, past and present, has been of long standing but I find little reason for it. Both species occur in Louisiana and they can easily be distinguished in the living condition. I have also examined many specimens from the tropics and have collected in Panama, Peru, Bolivia and Argentina, and whereas *A. polytricha* is common in these areas, *A. auricula* is not found. The two species are structurally different not only in hair length, as Olive maintains, but in their internal organization as well.

My acquaintance with *A. cornea* is based chiefly on the examination of collections made by Rogers (**8**) in the Hawaiian and Marshall Islands, together with a few specimens from China, Hawaii, and the Marshall and Palau Islands found by others. Rogers points out the resemblance between *A. cornea* and *A. auricularis* (= *A. auricula*) but considers them "readily distinguishable," making the further comment that "the differences are much greater in living than in preserved material." My analyses of sectioned basidiocarps of *A. cornea* and *A. polytricha* confirm these observations.

Ductifera calcarea sp. nov.

Fructificatio firme gelatinosa, griseo-hyalina, primo pustulata dein coalescens, hymenio corrugato, margine determinata; nodulae conspicuae calcareae in hymenio; gloeocystidia lutea, 28–50 × 6–8 μ; hyphae conspicuae nodosae; basidia late ovata, 4-cellularia, 15.5–19 × 12–14 μ; basidiosporae cylindraceae, 14–18.5 × 6–7 μ.

Lowy B95, TYPE, 1–XII–1958, 216 km east of Cochabamba. On dicot wood.

Fructification firm-gelatinous, greyish-white when fresh, arising as pustules, becoming effused with determinate margins, with calcareous nodules up to 1 mm broad; drying to brownish, horny patches; hymenium with abundant yellow clavate gloeocystidia, 28–50 × 6–8 μ; basidia mostly ovate, 4-celled, becoming cruciate-septate, 15.5–19 × 12–14 μ; epibasidia subcylindric, predominantly short and broad, 20–30 × 4–5 μ; basidiospores plump-cylindric, rarely slightly curved, 14–18.5 × 6–7 μ; germinating by repetition or by germ tube.

The new species is very close to *Exidia nucleata* (Schw.) Burt and would easily be mistaken for the latter upon macroscopic examination because of the presence of distinctive calcareous nodules. The unexpected occurrence of gloeocystidia raises a question regarding the affinities of the new fungus. It appears to be intermediate between the gloeocystidiate *Bourdotia* and *Exidia*, which is characterized at least in part by its determinate margins. The use of *Ductifera* Lloyd as redescribed by Wells (**10**) provides a satisfactory basis for the inclusion of certain tremellaceous species that have heretofore been ill-defined. Its acceptance makes *Gloeotromera* Ervin superfluous.

EXIDIA NUCLEATA (Schw.) Burt, Ann. Mo. Bot. Gard. **8**: 371. 1921.

The Bolivian collection dries to a barely visible film, making the calcareous nodules more prominent. Dendrophyses were abundant, measuring 20–30 × 1.5–2 μ. In other respects this differs little from temperate and tropical material that I have seen.

Singer B695, 7–II–1956, Prov. Nor-Yungas, Dpto. La Paz. On dicot wood.

PSEUDOHYDNUM GELATINOSUM (Fr.) Karst. var. **paucidentata** var. nov.

Fructificatio alba, elastico-gelatinosa, sicca cornea, dentata, dentibus ad 1 mm alt., non frequentibus; basidia 2–4-septata, 12.5-15 × 9–11 μ; basidiosporae subglobosae, 7-9(-10) × 6-8.5 μ.

Fructification thin, white, rubbery-gelatinous, drying to a tough, translucent film; spines widely scattered, simple to forked, up to 1 mm long; basidia 12.5–15 × 9–11 μ, 2–4-septate; basidiospores subglobose, 7–9(–10) × 6–8.5 μ.

Other collections of this species that I have seen from the tropics and elsewhere have been densely spiny, the entire fruiting body drying dark brown. The Bolivian fungus remains almost unchanged in color and the widely scattered spines make it distinctive. The basidiospores are also larger than normal, 5–7 μ being the usual range of size.

Singer B966, TYPE, 7–II–1956, San Jerónimo, Prov. Nor-Yungas, Dpto. La Paz. On dicot wood.

TREMELLA FUCIFORMIS Berk., Jour. Bot. Kew Misc. **8**: 277. 1856.

This differs in minor ways from other tropical material I have seen. The dull-white, gelatinous fruiting body has fewer lobes and the basidiospores are somewhat larger than normal, measuring 6–8.5 × 5–7 μ.

Singer B813, 1–II–1956, Prov. Nor-Yungas, Dpto. La Paz. On bark of dicot, moss-covered wood.

TREMELLA LUTESCENS Fries, Syst. Myc. **2**: 213. 1822.

The bright orange to orange-yellow tough-gelatinous fructifications were typical of the species. Conidia were abundant and larger than normal, measuring 3–4.5 × 3–4 μ.

Lowy B78, Siberia, 29–XI–1958, Prov. Valle Grande, Dpto. Cochabamba. On dicot wood; *Lowy, B92,* 1–XII–1958, 216 km east of Cochabamba. On dicot wood.

ARRHYTIDIA INVOLUTA (Schw.) Coker var. **boliviensis** var. nov.

Fructificatio ceraceo-gelatinosa, resupinata, sicca aurantio-brunnea; dikaryoparaphyseae cylindraceae, 50 × 3 μ; basidiosporae 1-3-septata, 14-17.2 × 5.5-6.5 μ; septa crassa.

Fructification waxy-gelatinous, resupinate, pustulate at first, becoming effused, drying reddish-brown; hymenium smooth; attached by conspicuous rooting bases; dikaryoparaphyses long-cylindrical, up to 50 × 3 μ; hymenial conidia up to 5 μ in diam., arising from septate conidiophores; basidiospores also producing conidia 2.5–3.5 × 2–3 μ; probasidia cylindrical, up to 58 × 4.5–5 μ; epibasidia up to 52 × 3 μ; basidiospores becoming 1–3-septate, 14–17.2 × 5.5–6.5 μ, septa thick.

Lowy B90, TYPE, 1–XII–1958, 216 km east of Cochabamba. On rotten dicot wood.

With regard to texture, rooting bases and basidiospore measurements, this is in close agreement with *A. involuta,* but in my opinion the thick-septate basidiospores, dikaryoparaphyses and distinctive conidiophores make the consideration of varietal status desirable. Several basidia with aberrant septation were observed, the septa occurring at epibasidial bases.

CALOCERA CORALLOIDES Kobayasi, Sci. Rep. Tokyo Bunr. Daig. B **4**(74): 225. 1939.

Fructifications tough-gelatinous, erect, digitate, cylindrical to slightly flattened, united below in a common base; apex pointed to blunt; up to 5 mm in height, 0.5–1 mm in diam.; pale orange above, reddish brown below, becoming darker on drying; probasidia 25–35 × 3–4 μ; epibasidia up to 14–25 μ; dikaryoparaphyses cylindrical, 23–42 × 2–2.5 μ; basidiospores ovoid, becoming 1-septate, 8.5–11 × 4–5 μ with prominent apiculus; conidia globose to subglobose, 2–3 μ diam.; no clamp connections observed.

Singer B1279, 19–II–1956, Carmen Pampa, Prov. Nor-Yungas, Dpto. La Paz.

To the best of my knowledge, this species has previously been reported only from Japan. The Bolivian collection agrees in essential detail with Kobayasi's (2) original diagnosis.

Dacryopinax Indacocheae sp. nov. FIG. 1

Fructificationes gelatinosae, stipitatae vel substipitatae, pileatae vel foliaceae, pallido-brunneae, siccae corneae, brunneae, hymenio inferiore, unilaterali; abhymenio tomentoso, sicco albido; hyphae enodosae; conidia frequentia; basidiosporae curvulo-cylindraceae, 2-cellulae, (8.5–)9-10(–11) × 3–3.5 μ.

Singer B1642, TYPE, 7–III–1956, Prov. Vaca Diez, Dpto. Beni, on dicot wood; *Singer B2512*, 5–IV–1956, Prov. Madre de Diós, Dpto. Pando, on dicot wood; *Singer B1953*, 14–III–1956, Prov. Vaca Diez, Dpto. Beni.

Fructifications rubbery-gelatinous, tan, stipitate to substipitate and pileate to foliose with multiple lobes, up to 2 cm in height and 3 cm broad; drying horny and light brown; hymenium inferior, unilateral, smooth; abhymenium tomentose, whitish when dry; hairs simple or branching, made up of inflated, thick-walled catenate cells; hyphae without clamp connections; probasidia cylindrical to subclavate, tapering below, 25–35 × 3–4.5 μ; dikaryoparaphyses cylindrical, 20–30 × 2–2.5 μ; conidiophores phialide-like, conidia 4–5 × 2–2.5 μ; basidiospores cylindrical to curved-cylindrical, predominantly 1-septate, (8.5–)9–10(–11) × 3–3.5 μ, septum thick; basidiospores producing subglobose conidia 2–2.5 μ diam. This species is named in honor of Dr. Eduardo F. Indacochea of Lima, Perú.

Variations in external appearance of the fructifications of this species clearly demonstrate one of the intermediate characteristics that makes interpretation of some members of the group puzzling. The photograph (FIG. 1) shows two fruiting bodies of the type collection, one small, distinctly stipitate and pileate, the other larger and foliose with a short stipe. Microscopically these are indistinguishable but had they represented different collections, their identity might not at least superficially have been apparent.

The following new combination is proposed:

Dacryopinax Imazekiana (Kobayasi) comb. nov.

Guepinia imazekiana Kobayasi, Sci. Rep. Tokyo Bunr. Daig. B **4**(74): 220. 1939.

A consideration of Kobayasi's (2) description and figure makes it clear that this species should be transferred to *Dacryopinax*.

Dacryopinax yungensis sp. nov. FIG. 2

Fructificatio cartilagineo-gelatinosa, stipitata, viva lutea, sicca cornea, fusco-brunnea; caulis 1.5 cm alt., multi-pilosa, pileo cum papillis conspicuis; hyphae nodosae; hymenio unilaterali; basidia bifurcata; probasidia 28–46 × 3–3.5 μ, epibasidia ad 45 μ; basidiosporae curvulo-cylindraceae, 10.6–13 × 5–6 μ, 3-septata.

Singer B614, TYPE, 28–I–1956, Cataratas "San Juan," Prov. Nor-Yungas, Dpto. La Paz. On dicot wood.

Fructification tough-gelatinous, stipitate, pileate, yellow when fresh, drying to red-brown; horny; stipe central, 2–5 mm diam., up to 1.5 cm tall, densely hairy, sulcate; pileus up to 1.5 cm broad with shorter hairs on abhymenium; hymenium with coarse papillae, rounded to almost spiny; hyphae with clamp connections, some bulbous hyphae found; probasidia 28–46 × 3–3.5 μ; basidia bifurcate, slender; epibasidial part up to 45 μ long; basidiospores curved-cylindrical, 10.6–13 × 5–6 μ, becoming 3-septate.

The genus *Dacryopinax* was established by Martin (**5**) to include members of the Dacrymycetaceae with stipitate, pileate fructifications and unilateral, inferior hymenia borne "on a patellate or flabelliform receptacle." Three species were recognized, including *D. elegans* (Berk. & Curt.) Martin, *D. fissus* (Berk.) Martin and *D. Spathularia* (Schw.) Martin. Until the present no new species have been described. This distinctive pileate, long-stipitate species is closest to *D. elegans* (Berk. & Curt.) Martin but differs from it in being yellow when fresh and is more densely tomentose, centrally stipitate and having a papillate hymenium. Kobayasi (**2**) has described a centrally stipitate *Guepinia* from Japan but it is apparent from his publication that the species is distinct from the Bolivian fungus. The presence of papillae in *D. yungensis* is also notable since as far as I can determine, no other papillate *Dacryopinax* has been reported.

DACRYOPINAX SPATHULARIA (Schw.) Martin, Lloydia **11**: 16. 1948

This species appears to be widely distributed throughout Central and South America. I have collected it in Panama, Peru, Bolivia, and Argentina; it is one of the commonest gelatinous fungi of these regions. From my observations of many collections, including North American material, I find considerable variation in external appearance. Some fructifications are simply spathulate on the usual tomentose stalk while others approach a lobed-flabelliform condition. Pigmentation is variable, yellow and orange predominating. Microscopically these variants are not significantly different.

Singer B2004, 16–III–1956, Guayaramerín, Prov. Vaca Diez, Dpto. Beni, on dicot wood; *Singer B-1157*, 13–II–1956, Charobamba, Prov. Nor-Yungas, Dpto. La Paz, on dicot wood; *Lowy B107*, 3–XII–1958, Siberia, Prov. Valle Grande, Dpto. Cochabamba, on dicot wood.

Type material of the new species is deposited in the mycological herbaria of Louisiana State University, Baton Rouge, the National Fungus Collections, Beltsville, Md., and the Universidad Nacional de Tucumán, Tucumán, Argentina.

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THE EFFECT OF VARIOUS SUBSTANCES ON THE OXYGEN UPTAKE OF RHIZOPUS ORYZAE

YOUSEF AL-DOORY [1]

There has been no report on the oxidative metabolism of *Rhizopus* and the object of the present investigation is to study the effect of various substances on the oxygen uptake of *Rhizopus oryzae* as a representative of this genus.

MATERIALS AND METHODS

The fungus, *Rhizopus oryzae,* was grown at room temperature in 25 × 150 mm culture tubes on Sabouraud's dextrose agar medium and harvested when it was 8–10 days old. Mycelial growths were washed from the surface of the media and collected in sterile centrifuge tubes. The suspensions were agitated and the fluid portion containing the spores was used. The spores were washed twice by centrifugation and then the packed cells diluted 1:10 in 0.067 M phosphate buffer, pH 7.

Determinations of the oxygen uptake were made in an atmosphere of air at 37° C using the Warburg constant volume respirometer following the techniques described by Umbreit et al. (**4**). The final volume of liquid in each Warburg flask was 2.0 ml: one-half ml of the cell suspension, 1 ml of the buffer solution, and one-half ml of substrate in the sidearm. All substrates were added in a final concentration of M/300.

Results represent the average of at least two separate determinations and are tabulated as the per cent inhibition or stimulation of oxygen uptake in the experimental flasks as compared to that of the control flasks. None were considered significant unless the deviation from the control for each substrate used was more than 10 per cent.

RESULTS

Tricarboxylic Acid Cycle Intermediates. TABLE I represents the oxygen uptake of the fungus with the Krebs Cycle intermediates as sub-

[1] The author wishes to express appreciation to Drs. Howard Larsh and George Cozad for their assistance during this investigation. This work was supported in part by a grant (E-1292) from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, U. S. Public Health Service.

TABLE I

EFFECTS OF TRICARBOXYLIC ACID CYCLE INTERMEDIATES ON OXIDATIVE METABOLISM OF RHIZOPUS ORYZAE

Substrate (M/300)	Oxygen uptake (μl) with substrate	Oxygen uptake (μl) without substrate	Stimulation* %
Pyruvic acid	211.2	141.6	49.2
Oxalacetic acid	112.2	71.7	56.5
Citric acid	96.5	71.7	34.6
Cis-aconitic acid	186.3	160.7	15.9
Isocitric acid	185.7	155.6	19.3
Oxalosuccinic acid	103.5	71.7	44.4
Alpha-ketoglutaric acid	164.6	147.3	11.1
Succinic acid	99.1	71.7	38.2
Fumaric acid	196.3	160.7	22.2
Malic acid	100.5	71.7	40.2

* 37° C, pH 7.0. Totals for five hours.

TABLE II

STIMULATORY EFFECTS OF CERTAIN AMINO ACIDS ON OXIDATIVE METABOLISM OF RHIZOPUS ORYZAE

Substrate (M/300)	Oxygen uptake (μl) with substrate	Oxygen uptake (μl) without substrate	Stimulation* %
Glycine	153.0	111.7	37.0
Phenyl glycine	168.9	131.8	28.2
L-alanine	356.1	155.6	128.8
DL-alpha alanine	273.8	148.9	83.9
L-valine	157.4	122.1	28.9
D-valine	140.2	114.4	22.6
DL-valine	170.9	131.8	22.1
L-leucine	172.0	131.8	30.5
D-leucine	167.6	139.0	20.6
L-isoleucine	151.7	132.0	14.9
DL-serine	231.7	168.6	37.4
L-threonine	166.7	139.0	19.9
L-phenylalanine	151.5	113.7	33.2
L-tyrosine	191.5	148.9	28.6
L-tryptophane	154.4	131.8	17.1
DL-tryptophane	159.0	141.6	12.3
L-cysteine	159.2	132.0	20.6
L-methionine	174.1	135.5	28.9
D-methionine	152.3	113.7	33.9
DL-methionine	159.2	134.1	18.7
L-aspartic acid	172.6	135.3	27.6
D-aspartic acid	144.8	127.0	14.0
L-glutamic acid	296.9	131.8	125.3
DL-histidine	209.7	160.7	29.9
L-proline	288.8	104.1	176.9**
DL-proline	154.9	88.0	176.1**
DL-citrulline	153.8	135.4	13.6
L-arginine	253.2	132.0	91.8

* 37° C, pH 7.0. Totals for five hours.
** Totals for three hours.

strates. All of them stimulated the oxygen uptake, the greatest amount occurring with oxalacetic acid, followed by pyruvic acid, oxalosuccinic acid, and malic acid with alpha-keto-glutaric acid producing the least amount of activity.

Amino Acids. Among the amino acids tested in this study the L-forms produced more oxygen uptake activity than either the D- or the DL-forms. TABLE II presents the results obtained from this study. L-proline, DL-proline, L-alanine, and glutamic acid produced the greatest amount of activity, while allyl glycine, D-alanine, D-phenylalanine, and L-cystine did not show a significant amount of activity.

TABLE III

EFFECTS OF CARBOHYDRATES ON OXYGEN UPTAKE OF RHIZOPUS ORYZAE

Substrate (M/300)	Oxygen uptake (μl) with substrate	Oxygen uptake (μl) without substrate	Stimulation* %
Pentoses			
Xylose	136.8	71.7	90.8
Arabinose	145.7	134.7	8.2
Hexoses			
Glucose	383.7	284.7	34.8
Mannose	318.9	137.5	131.9
Fructose	231.5	137.5	68.4
Galactose	254.8	137.5	85.3
Disaccharides			
Lactose	176.2	141.6	24.4
Maltose	238.7	160.7	48.5
Sucrose	153.3	144.9	5.8
Polysaccharides			
Starch	169.4	135.4	25.1
Glycogen	274.7	160.7	70.9

* 37° C, pH 7.0. Totals for five hours.

Carbohydrates. Xylose was used oxidatively by the fungus, while L-arabinose did not show a significant amount of activity. Results are presented in TABLE III.

All of the hexoses, disaccharides, and polysaccharides tested, with the exception of sucrose, caused a definite increase in the respiratory rate. Mannose was the most effective, with galactose, glycogen, and fructose following in the order named.

DISCUSSION

The respiration of *Rhizopus oryzae* appears to be stimulated in general by the amino acids and carbohydrates used in this study. It appears to be similar to *Spicaria violacea* in that the oxygen uptake of the latter was stimulated by glucose (1). The same was found from

this study with *R. oryzae*. McCallan et al. (2) reported that the oxygen uptake of *R. nigricans* was increased by some of the fungicides in the presence of one percent sucrose; however, this carbohydrate showed no significant influence on the respiration of *R. oryzae*. The respiration of *R. oryzae* in the presence of L-leucine resembles the results obtained by Schade and Thimann (3) with the water mold, *Leptomitus lacteus*.

The results of this study suggest that at least part of the tricarboxylic acid cycle is involved in the metabolism of *R. oryzae*.

SUMMARY

The effect of various tricarboxylic acid cycle intermediates, amino acids, and carbohydrates on the respiration of *Rhizopus oryzae* was studied by using conventional manometric techniques.

All of the tricarboxylic acid cycle intermediates stimulated the oxygen uptake of the fungus.

The L-forms of the amino acids produced more oxygen uptake than either the D- or the DL-forms and the greatest amount of activity was produced by L-proline.

All of the carbohydrates tested stimulated the oxygen uptake of the fungus with the exception of L-arabinose and sucrose. Mannose produced the highest activity.

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THE FUNGUS FLORA OF CATTLE FEEDS[1]

R. D. Bonner and C. L. Fergus[2]

Cattle feeds consist of vegetable matter which may serve as a substrate for various molds or fungi. The fungus flora of cattle feeds, especially silage, has not been determined. The identification of such fungi is important in problems of storage and preservation. In addition, certain fungi, such as *Aspergillus* spp. and *Mucor* spp., are known to cause disorders in animals as a result of inhaling the spores or ingesting moldy feeds. Respiratory, nervous and intestinal disturbances have been found to develop, along with internal mycoses following the effects of allergins, or toxins, or both (**3**, **7**, **8**). The possibility that other fungi may do this lends importance to the study. Conflicting interpretations have been placed upon the role of fungi, particularly molds found on feeds, in disease associations. Much of the confusion may stem from the fact that very few attempts have been made to determine accurately the identity of the fungi involved, or to determine experimentally their effects on cattle under controlled conditions.

The present study involved the identification of the fungus flora of cattle feeds, some of which had been fed to cattle that later sickened. This investigation required the adaptation and development of techniques to ensure isolation of all micro-organisms (except bacteria) from the external and internal portions of feeds of different composition and physical consistency, and their taxonomic study.

MATERIALS AND METHODS

The feeds studied had been collected or sent to the Veterinary Research Center from various regions of Pennsylvania. They were silages, dried forages and dried grains that had been fed to cattle that subsequently became ill, and hence were suspected as having caused the ill-

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[2] Appreciation is hereby expressed to Dr. H. W. Dunne, Professor of Veterinary Science, who suggested the study and supplied the feed samples studied.

ness. For comparison, silages and other feeds, designated as "non-suspected," were also studied.

All silages had been preserved with sodium metabisulphite. Before attempting to isolate fungi from them, all feeds were analyzed for their pH, moisture content, extent of deterioration, presence of micro-organisms other than fungi, and the presence of soil or debris. The per cent moisture content was determined by drying in an electric oven at 80° C and expressed as a percentage of the oven-dry weight of the feed. A glass-electrode pH meter was used to determine the pH of samples prepared by adding 100 ml of distilled water to 50 grams of feed.

Visual inspections were made of individual samples to determine the types of materials present. Further examination was made with the aid of a stereoscopic microscope. Small portions of samples and dilutions were examined with the compound microscope to detect the presence of and identify actively developing fungi as well as other micro-organisms.

To isolate fungi in pure culture, 1 g samples were placed in Erlenmeyer flasks containing 100 ml of sterile distilled water and shaken vigorously. Ten-ml portions of the liquid were withdrawn by pipette and diluted serially to 1:100, 1:1000 and 1:10,000. In preparing samples for determining internal fungi, feeds containing grains and seeds were surface sterilized in 1:1000 mercuric chloride for 15 minutes, then rinsed in sterile distilled water. The grains and seeds were crushed or ground under aseptic conditions and suspended in 10 ml of sterile distilled water. Surface-sterilized materials were also placed directly on hardened malt-extract agar.

To ensure isolation of organisms with varying nutritional requirements, various culture media were employed. They were: malt extract (**6**), malt-salt (**4**), Littman oxgall (**10**), potato-dextrose (**6**), and Czapek-Dox (**13**) agars. All media were autoclaved for 15 minutes at 15 pounds.

Inoculations of plates were made by pipetting 0.5 ml of each dilution series onto the hardened surfaces of the five media in petri dishes. Cultures were prepared in quadruplicate and incubated at 20° for 5 to 15 days. At 5-day intervals, a complete set of cultures was removed to determine the number of colonies developing and to transfer individual fungi to test tubes of agar media for identification (**2, 5, 9, 11, 12, 13, 14**). Malt-extract agar was used as a general medium for maintaining stock cultures of the filamentous fungi. Tentative and even final identifications were usually made on this medium as soon as adequate sporulation

had occurred. Special media were used for identifications of *Aspergilli* and *Penicillia* (**12**, **13**), and the yeasts (**11**, **14**).

RESULTS

The composition, per cent moisture content, pH, and visual evidence of fungi are listed for each sample in TABLE 1. The pH of most samples was within the range which allows growth of a large number of fungi. The per cent moisture content varied considerably for samples of different composition. Most samples contained various micro-organisms, which could be detected by using the stereoscopic microscope. Mites and insects in various developmental stages were observed. Protozoa, helminths, and rotifers were frequently observed. None of them, however, were known animal parasites. In addition, the presence of

TABLE 1

ANALYSES OF FEEDS, INCLUDING THEIR DESCRIPTIONS, PER CENT MOISTURE CONTENT AND pH

Sample no.	Description	Per cent moisture content	pH
229	Chopped grasses, not moldy	40.5	5.6
230	Mixed grass, not moldy	38.3	5.3
252	Mixed grasses, very moldy	46.6	4.8
315	Mixed silage, extensive deterioration, very moldy	53.5	5.8
316	Soybean silage, very moldy	51.0	5.4
317	Blades of grass, advanced deterioration, pungent odor; moldy	47.8	4.3
318	Chopped vegetable materials, not moldy	46.6	4.8
328*	Mixed dry oats and corn, not moldy	28.0	6.5
329*	Dried grass, not moldy	18.6	7.2
330	Assortment of plant foliage, slightly moldy	28.5	5.7
331	Chopped corn leaves and stems, very moldy	42.0	4.9
388	Mixed grasses and assortment of plant materials, very moldy	49.3	5.1
415	Corn grain, broken cobs, very moldy	39.3	5.1
416	Alfalfa, very moldy, soil particles present throughout	48.7	5.6
417	Grass leaves and parts of various plants, not moldy	41.0	6.3
418*	Mixed grasses, not moldy	53.0	6.4
419*	Mixed vegetable materials, not moldy	56.0	6.4
434-A	Mixed corn leaves and soybean foliage, very moldy	51.0	5.2
434-B	Silage consisting of corn grain, fecal matter, very moldy	38.8	5.0
434-C	Oats, very moldy	28.0	6.3
434-D*	Oats, not moldy	32.6	6.0
434-E*	Ground feed, not moldy	42.6	6.3
78-S*	Hay ensilage, not moldy	53.5	5.7
79-S*	Hay ensilage, not moldy	50.6	6.2
84-S*	Soybean ensilage, not moldy	56.0	6.2
90-A*	Soybean ensilage, not moldy	56.3	6.0

* Feeds of a non-suspected classification.

TABLE 2

THE IDENTITY AND CLASSIFICATION OF THE FUNGI ISOLATED FROM TWENTY-SIX FEED SAMPLES

Classification	Feed-sample number
Phycomycetes	
Mucorales	
Mucoraceae	
Circinella umbellata v. Tiegh. & LeMonn.*	434-B[a]
Mucor flavus Bain.	329, 417, 434-C[a]
Mucor fragilis Bain.*	318, 328[b]
Mucor janssenii Lend.	419, 434-B[a]
Mucor racemosus Fres.*	388[a], 415[a]
Mucor ramannianus Moll.*	317[b]
Rhizopus arrhizus Fischer*	434-B[a], 434-E
Rhizopus nigricans Ehrenb. (= *Rhizopus stolonifer* (Fr.) Lind.)	316[a], 317[a], 328 417, 419, 434-A[a], 434-B[a], 434-C[a], 434-D, 434-E, 78-S, 84-S
Rhizopus nodosus Namysl.	318, 418, 434-C
Rhizopus oryzae Went. & Pr. Geerl.*	434-C
Mortierellaceae	
Haplosporangium decipiens Thax.*	317[b]
Ascomycetes	
Endomycetales	
Cryptococcaceae	
Candida albicans (Robin) Berkh.	78-S, 229, 318, 434-A
Candida humicola (Dasz.) Didd. & Lodd.	316, 331, 415, 434-E
Candida krusei (Cast.) Berkh.	230, 419
Cryptococcus albidus (Saito) Skinner	417, 434-B, 84-S[b]
Endomycetaceae	
Ascocybe sp.*	415
Geotrichum candidum Link ex Pers. emend Carmic.*	229, 318, 329, 330
Saccharomyces cerevisiae Hansen	315, 434-B, 434-E, 90-A
Aspergillales	
Gymnoascaceae	
Arachniotus terrestris Raillo	84-S[b]
Aspergillaceae	
Aspergillus chevalieri (Mangin) Thom & Church	329, 331[a], 434-C[a]
Aspergillus nidulans (Eidam) Wint.	315, 316, 434-E
Monascus purpureus Went	418, 78-S
Monascus ruber v. Tiegh.	252, 330, 419
Penicillium vermiculatum Dangeard	79-S[b]
Sphaeriales	
Chaetomiaceae	
Chaetomium olivaceum Cke. & Ell.	316[a], 388, 418

* Species isolated only from suspected feed samples.

[a] Fungi occurring as easily visible molds on feed samples.

[b] Species showing less than 100 colonies on malt-salt agar at dilutions of 1:100 and 1:1000. Species not listed in these categories produced 200 to 500 colonies.

TABLE 2—*Continued*

Classification	Feed-sample number
Deuteromycetes	
Moniliales	
Moniliaceae	
Aspergillus flavus Link ex Fr.	315[a], 316[a], 388, 415[a], 434-D
Aspergillus fumigatus Fres.	230, 252[a], 316[a], 317[a], 388[a], 416[a], 418, 434-A[a], 434-C[a]
Aspergillus niger v. Tiegh.	229, 230, 316, 317[a], 328, 330[a], 388[a], 417, 434-D, 434-E, 78-S, 79-S
Aspergillus oryzae (Ahlb.) Cohn	415[b], 434-C[a], 434-D[b]
Aspergillus terreus Thom*	317, 416
Aspergillus ustus (Bain.) Thom	331, 419
Cephalosporium kiliense (Gruetz) Hart.*	317[b], 434-C[b]
Chlamydomyces palmarum (Cke.) Mason*	252[a], 317[b]
Dactylium dendroides (Bull.) Fr.	416[a], 419
Gliocladium roseum Bain.	315[b], 418[b]
Masoniella chartarum G. Smith*	230[b]
Paecilomyces variota Bain.*	230, 318, 388[a], 434-A[a]
Penicillium chrysogenum Thom	419, 434-C, 84-S[b]
Penicillium crustosum Thom	229, 317[a], 418, 434-C
Penicillium cyclopium Westl.*	252[a], 316[a], 388, 434-A[a]
Penicillium duclauxi Delacroix*	229, 316, 318, 388
Penicillium expansum Link*	318, 330, 415[a], 417
Penicillium frequentans Westl.	317[a], 329, 418, 434-B[a], 78-S
Penicillium herquei Bain. & Sartory*	252[b]
Penicillium janthinellum Biourge	418[b]
Penicillium notatum Westl.*	388, 416
Penicillium oxalicum Currie & Thom	230, 330, 388[a], 418, 434-B, 434-D, 78-S
Penicillium purpurogenum Stoll	318, 434-C
Penicillium thomii Maire*	317[b], 331[b]
Scopulariopsis brevicaulis (Sacc.) Bain.	315[a], 318, 434-D
Trichoderma koningi Oudemans	415, 419
Trichoderma lignorum (Tode) Harz	318, 415[a], 417, 434-B[a]
Tritirachium album Limber*	230, 317, 331
Verticillium terrestre (Link) Lindau	229[b], 419
Tuberculariaceae	
Epicoccum granulatum Penz.*	331[a]
Fusarium moniliforme Sheldon	230, 315, 329, 330[a], 416[a], 418, 434-A[a], 434-B[a], 434-E, 90-A[b]
Dematiaceae	
Alternaria solani (E. & M.) J. & Gr.	252, 331, 419
Alternaria tenuis Nees ex Fr.	318, 417, 434-E[b]
Cladosporium elatum (Harz) Nannf.	318, 328, 416
Cladosporium herbarum Fr.	316[a], 331[a], 434-D
Pullularia pullulans Berkh.	418, 434-A[a]
Stemphylium botryosum Wallr.*	316
Sphaeropsidales	
Sphaeropsidaceae	
Phoma hibernica Grimes*	317, 416[a]
Actinomycetales	
Streptomycetaceae	
Streptomyces spp.	230, 316[a], 317[b], 328, 415[a], 418, 434-B[a], 434-C[b]

foreign debris, soil, and rodent and livestock fecal matter was observed.

The number of fungus colonies from one sample which developed on the different media varied considerably. Malt-salt agar proved to be the best for a quantitative determination of the number of colonies developing at the three dilution series, since most were restricted sufficiently to permit counting and isolation. Most fungi developed at a faster rate on malt-extract and potato-dextrose agars than on the other media. Littman oxgall allowed good growth of most of the Moniliaceae and Mucoraceae while limiting that of most other fungi. *Cephalosporium kiliense, Gliocladium roseum* and *Mucor fragilis* were isolated solely on this medium. Potato-dextrose agar supported the growth of practically all the fungi isolated; however, because of the rapid growth of certain species, pure-culture isolation and colony counting were very difficult on this medium even after only five days incubation.

Czapek-Dox agar was highly selective for the isolation of ascomycetes and deuteromycetes. Most of the yeasts failed to develop on the medium, and were rarely isolated on it. Most of the Mucorales failed to develop on this medium, and the few that did were strongly inhibited.

The fungi isolated from 26 feed samples represented 64 species, ranging through three classes, seven orders, and twelve families. Of the sixty-three species identified from "suspected" feed samples, thirty-eight of them were also isolated from "non-suspected" samples. *Arachniotus terrestris* and *Penicillium vermiculatum* were found only on non-suspected feeds.

Fourteen species were isolated from the internal surfaces of feed substrates; these were *Rhizopus oryzae, Arachniotus terrestris, Aspergillus nidulans, A. ustus, Monascus purpureus, Penicillium frequentans, P. thomii, P. vermiculatum, Chaetomium olivaceum, Masoniella chartarum, Fusarium moniliforme, Alternaria solani, A. tenuis,* and *Stemphylium botryosum.* All of these species were encountered on the external surfaces of the same feeds from which they were recovered internally.

A classification and listing of the fungi isolated from all feed substrates is given in TABLE 2. All cultures have been deposited in the Fungus Culture Collection of the Department of Botany and Plant Pathology.

DISCUSSION

The present study was undertaken to further our knowledge of the fungus flora associated with various types of cattle feeds. The foremost objective was to determine the identity of the fungi present in both sus-

pected and non-suspected feeds. If fungi with known pathogenic or toxic abilities were found, their influence in disease conditions might be ascertained. Identification required the application of various isolation techniques, and the use of taxonomic literature.

With the range of synthetic and natural media used, it is likely that any viable fungus present in the feeds would have developed on one or more. However, it is recognized that other limiting factors were not considered, such as temperature of incubation. Only one temperature was used, i.e., 20° C. It was selected because it is the optimum for the greatest number of fungi. In addition, the thermophilic micro-organisms developing in the silo may have died prior to the attempted isolations, yet may have been responsible for the toxic state of the feed sample.

It was also realized that organisms other than fungi or factors not generally associated with fungus species might be involved in the induction of pathologic or toxic conditions. Analyses and observations of the external surfaces of materials, hand sections, and washings revealed micro-organisms other than fungi. Similarly the pH, per cent moisture content, and the physical makeup of the feeds were determined, but these factors do not appear to be significant.

Quantitative counts of colonies from moldy feeds far exceeded the numbers obtained from non-moldy feeds. On the other hand, the variety of fungus species isolated from non-moldy feeds was very great. Not all of the fungi isolated from diluted washings would be developing actively or extensively in or on the feed. In addition, some of the spores could have fallen onto the sample from the air.

Many of the fungus species isolated in the present study have been reported to be associated with pathological conditions in farm animals (**1**). Their identification represents the first step in elucidating their possible role in causing diseases of livestock. Their exact role needs to be investigated by an animal pathologist, by feeding to animals silages inoculated with a single named fungus isolate, or exposing animals to spores as possible allergens. Many associations of molds with livestock diseases have been assumed, and not proven. More definite proof is necessary than is now at hand.

SUMMARY

The fungus flora of 26 feed samples, 16 of which had histories associated with disorders of livestock, has been determined and compared to that of non-suspected feeds. Visual and stereomicroscopic analyses of the feed substrates showed that the samples were generally heavily contaminated with molds, other micro-organisms, soil, feces, and debris.

Special techniques of isolation and the employment of a wide range of natural and synthetic media, viz., malt-salt, malt-extract, Czapek-Dox, Littman oxgall and potato-dextrose media permitted isolation of a variety of fungi. The formation of discrete colonies on malt-salt generally allowed quantitative determinations of the number of colonies of fungi from dilutions of washings as well as easy isolation in pure culture. Littman oxgall agar permitted the isolation of several species not encountered on the other media. Malt-extract and potato-dextrose agars generally permitted rapid growth of the molds. Czapek-Dox was highly specific for isolation as well as culture of members of the Aspergillaceae and certain Moniliaceae.

Sixty-four species representing 3 classes, 7 orders and 12 families were found on the external surfaces of the feed materials. Only 14 of this number were found to occur both externally and internally. Of the 64 species identified, 63 were obtained from suspected samples and 38 of these were isolated also from non-suspected feeds. The following species isolated have known or suspected pathogenic or toxic abilities in cattle or other livestock: *Mucor flavus, M. racemosus, Rhizopus arrhizus, R. nigricans, Haplosporangium decipiens, Candida albicans, C. humicola, C. krusei, Cryptococcus albidus, Aspergillus chevalieri, A. flavus, A. fumigatus, A. nidulans, A. terreus, Monascus purpureus, Penicillium expansum, Chaetomium olivaceum, Cephalosporium kiliense, Paecilomyces variota, Scopulariopsis brevicaulis, Trichoderma koningi, Fusarium moniliforme, Cladosporium herbarum, Pullularia pullulans,* and *Streptomyces* spp.

Members of the Aspergillaceae were predominant in the flora of 25 of the feeds studied.

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OBSERVATIONS ON GYMNOASCACEAE. VI. A NEW SPECIES OF ARACHNIOTUS

HAROLD H. KUEHN AND G. F. ORR

(WITH 19 FIGURES)

The genus *Arachniotus* was erected in **1893** by Schroeter to include those Gymnoascaceae which possessed a peridium composed of uniform, thin-walled hyphae. Included in the genus at that time were *A. candidus* (Eidam) Schroeter, *A. ruber* (Van Tiegh.) Schroeter and *A. aureus* (Eidam) Schroeter. Recently, Kuehn (**1957**) reviewed the genus *Arachniotus* while describing a new species, *A. reticulatus*. At that time Kuehn suggested that the three original species of *Arachniotus* might be removed from the genus since bibliographic evidence indicated that a peridium as such was lacking in these three species. However, since type material of these species did not seem to be available, no definite decision was made. Certain fungi do fit the generic limits erected by Schroeter, even though the original three species might not. *Arachniotus reticulatus* Kuehn has a definite peridium as does another fungus, RSA 202, which Benjamin (**1956**) described and illustrated but did not name. The purpose of the present paper is to describe a new species of *Arachniotus*.

Arachniotus flavoluteus sp. nov.

Cleistotheciis presentibus, plus minusve globosis, in agaro YpSs flavis aut luteis, 85–600 μ diam. Hyphis peridii duobus typis: et laevibus, septatis, muris laevibus, 1.1–1.7 μ diam. et hyphis pyriformibus usque ad 13 μ diam. ad fines ventricosus. Ascis ovoideis, hyalinis, 8.2–9.9 × 8.4–12 μ diam., muris evanescentibus, octosporis, adsociatis. Ascosporis colore flavo-luteis tinctis, oblatis, 2.2–4.4 × 3.9–7.7 μ, muris laevibus, marginibus circumvallatis, nonnunquam reticulatis circum axes spororum singulos, marginibus magnis 0.3–0.8 μ. Hyphis sterilibus cum granulis flaveo-luteis, 1.4–2.5 μ diam. Hyphis ad finem ventricosos presentibus. Sporis accesoriis absentibus.

Ascocarp present, more or less spherical, shades of orange-yellow on YpSs agar, 85–600 μ diam. Peridial hyphae of two types, smooth, septate, thin-walled, 1.1–1.7 μ diam., and racquet hyphae with inflated areas up to 13 μ diam. Asci ovoid, 8.2–9.9 × 8.4–12 μ, hyaline, wall evanescent, 8-spored, with the spores adhering in conglomerate groups,

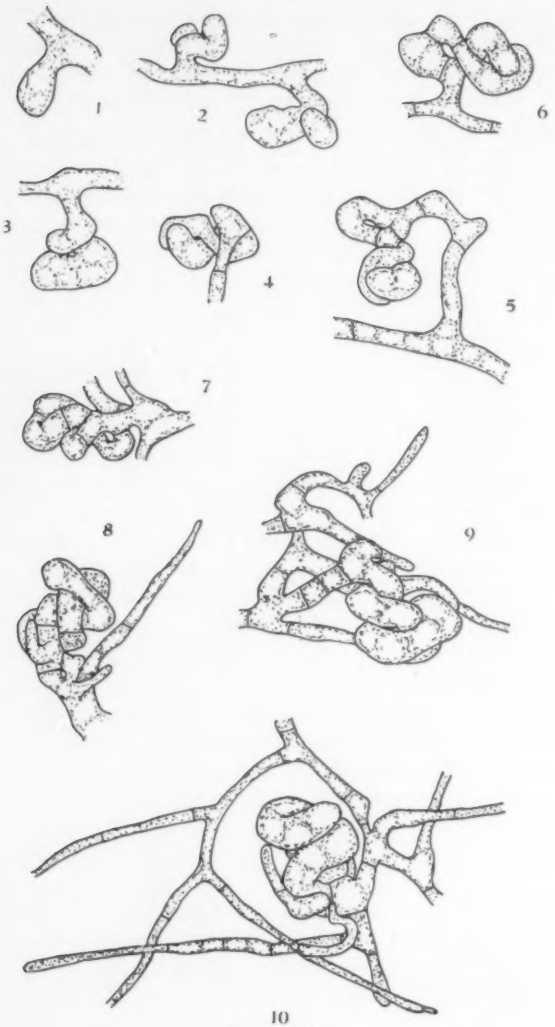
Ascospores shades of yellow-orange, oblate, 2.2–4.4 × 3.9–7.7 μ, smooth-walled, with one elevated rim, occasionally fluted so as to appear reticulate, around each axis of the spore; rims 0.3–0.8 μ diam. Vegetative hyphae hyaline or with yellow-orange colored granules, 1.4–2.5 μ diam.; racquet mycelium present. No accessory spore type present.

Three strains of this species have been isolated and studied. The type culture, NRRL 1243, was isolated from Arizona soil by C. W. Emmons who sent it to the Northern Utilization Research and Development Division, Peoria, Illinois, in October, 1941. Culture O-581 was isolated by G. F. Orr from lizard dung collected three miles east of Maricopa, California. Strain O-483 was isolated by G. F. Orr from lizard dung, Blythe, California.

Colonies on YpSs agar grow slowly at room temperature (23–28° C) and attain 2 inches diam. in 18 days. The colonies are pasty and hyaline at first; aerial cottony growth is white, then yellow as the asci develop, orange-yellow in about 20 days and finally shades of orange to deep orange. The amount of aerial mycelium may be great or small depending upon the particular strain. Ascocarps may develop in the aerial mycelium or on restricted hyphal areas in strains where little or no aerial mycelium develops. The ascocarps may or may not develop in concentric rings. Reverse of the colony is colorless to very pale yellow, or may be orange. The ascus clumps may be identified initially as discrete yellow knots which develop throughout the colony giving to the colony its characteristic color. These knots of asci appear in about 6 days and mature within 48 hours after their appearance. A slight dermatophyte-like odor is present but it is not pronounced. The vegetative hyphae are often hyaline, but may form ethanol soluble, yellow-orange granules or incrustations in the walls. Such granules are especially evident in water mounts. An exudate is produced on the surface of the colony. This exudate is colorless at first, but becomes red-orange.

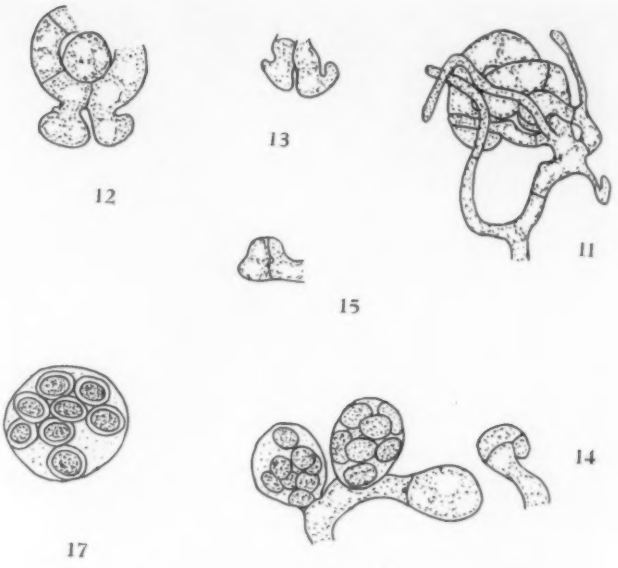
Colonies on Sabouraud's agar are white, cottony, with growth so luxuriant that the Petri plate is almost filled in a vertical direction. Reverse of the colony is colorless, becoming slightly pink with age. Asci appear in 13 days as white tufts on the surface of, and also within, the cottony growth. The tufts become yellow and mature asci are present within 2–3 days. With age the groups of asci become dark yellow. Often they are so numerous as to give the entire cottony mycelium a yellow appearance. An odor, typical of many of the Gymnoascaceae, is produced quite strongly.

On Czapek's agar the colonies are restricted, very thin and white.



FIGS. 1–10.

FIGS. 1–10. *Arachniotus flavoluteus*. FIG. 1. A very young club-shaped branch arising from the parent hypha. FIG. 2. A vegetative hypha bearing two young coils. Each club-shaped branch becomes an antheridium which forms the ascogonium as a lateral branch. FIG. 3. An abnormally long initial which has not yet branched to form two gametangia. FIGS. 4–6. Stages in coiling of gametangia.

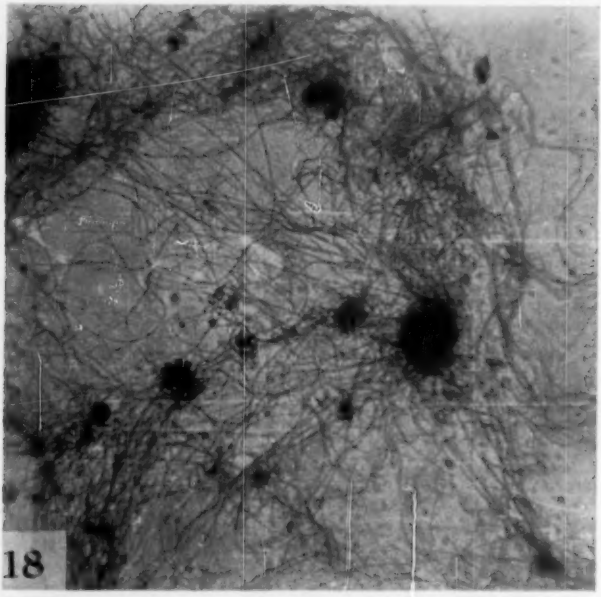


FIGS. 11–17. *Arachniotus flavoluteus*. FIG. 11. A typical older coil. FIG. 12. A coil seen from above, with young ascogenous hyphae arising from a septate ascogonium. FIGS. 13–15. Stages in crozier formation. FIG. 16. An immature ascus and two mature asci at the apex of an ascogenous hypha. FIG. 17. A mature ascus with ascospores. All figures × 1300.

Reverse of the colony is colorless. Asci appear as scattered yellow tufts on the surface of the agar, and constitute the entire aerial growth of the fungus.

Ascus formation is initiated by two morphologically similar branches which arise on the same hypha and proceed to spiral about one another. The parent hypha on which the two initials arise is a short lateral branch of another hypha. This short branch either bifurcates into two initials of similar size and shape (FIGS. 2, 4–6), or it may form a terminal, straight, club-shaped antheridium and a lateral ascogonium which

FIGS. 5–6 show the septum which delimits one of the initials. FIG. 6 also shows a septate ascogonium. FIGS. 7–8. Vegetative branches arising from the base of the coil, as well as from the parent hyphae below the coil. FIG. 9. An older coil, showing both antheridium and ascogonium to be septate. FIG. 10. Septate gametangia with vegetative branches arising from the base of the coil and also from the parent hypha. All figures × 1300.



FIGS. 18–19.

coils 2 or 3 times about the antheridium (FIG. 10). Each initial becomes separated by a septum from the parent hypha. No evidence was obtained which would indicate that fusion of the gametangia had taken place. The ascogonium becomes several-septate, while the antheridium may or may not become once- or twice-septate (FIGS. 7, 8, 11). From some of the ascogonial cells ascogenous hyphae arise. Sometimes these hyphae remain short and immediately produce croziers (FIG. 12), or they may elongate considerably prior to forming croziers. The penultimate cell may form an ascus, but more often it grows out to form a continuation of the ascogenous hypha as does the cell resulting from the fusion of the antepenultimate and ultimate cells. In this manner an extensive group of ascogenous hyphae is formed with asci ultimately arising from the penultimate cells of croziers.

Thin vegetative hyphae appear from the base of the gametangia, or from the parent hypha near the base of the coil, and grow up around the initials (FIGS. 8, 10, 11). These slender hyphal branches usually become extensive, keeping pace with the growth of the ascogenous hyphae, with the result that the asci develop within these hyphae to form an ascocarp. The peridium is composed of several layers of hyphae with a total width of about 10 μ. However, many smaller clusters of asci are formed completely devoid of a peridium. The asci are then in the same condition as is found in the genus *Pseudoarachniotus*. Strains O-581 and O-483 rarely have this naked condition of the asci, while it is quite frequently present in the smaller ascus groups of strain NRRL 1243. Although a certain amount of confusion might result from this variable condition, the larger groups of asci never lack a peridium and this enables the investigator to place the fungus within the genus *Arachniotus*.

Considerable strain variation occurs. Ascocarps vary from large, 170–600 μ diam., in O-483, to small, 87–300 μ diam., in O-581 and 95–335 μ diam. in NRRL 1243. However, small ascocarps, 80–170 μ diam., are infrequently present in O-483. Ascospores may be large, 3.3–4.4 $\times$ 4.4–7.7 μ in O-483 or smaller, 3.3 $\times$ 5.5 μ in NRRL 1243 and 3.3 $\times$ 5.0 μ in O-581. However, the major difference among these strains lies in the size of the inflations on the racquet hyphae in the peridium. In O-581 racquet hyphae are often completely lacking from the peridium, in NRRL 1243 such hyphae are present but with small

FIGS. 18, 19. *Arachniotus flavoluteus*. FIG. 18. Clusters of asci, some minus a peridium, others with a peridium, $\times$ 110. FIG. 19. Ascospores, showing the rims which are present on each axis. The fluted nature of the rim is apparent on at least one spore, $\times$ 1379.

enlargements, up to about 3.0 μ diam. In O-483 the peridium has many racquet hyphae with large inflations up to 13 μ diam.

Arachniotus flavoluteus may easily be distinguished from the other known species in this genus. *Arachniotus reticulatus* has ascospores which are spherical and reticulate, those of the as yet unnamed species of Benjamin, RSA 202, are pale yellow and smooth-walled, while ascospores of *A. flavoluteus* are oblate, orange-yellow and smooth-walled with rims. Furthermore, RSA 202 has a peridium with many spirally coiled appendages. If we consider *A. candidus, A. ruber* and *A. aureus* to be valid representatives of *Arachniotus,* then *A. flavoluteus* may also be easily distinguished from them as follows: the colony of *A. candidus* remains snowy white, as do the groups of asci throughout their development, *A. ruber* is brick red, *A. aureus* is golden yellow with spirally coiled peridial appendages, while *A. flavoluteus* is orange-yellow.

We would like to gratefully acknowledge the assistance of Dr. Tibor Benedek with the preparation of the Latin description.

SUMMARY

A new species, *Arachniotus flavoluteus,* is described and developmental morphology is presented. This new species can be distinguished from the other two available species of the genus, *A. reticulatus* and RSA 202, by ascospore characteristics. The new species is also easily distinguishable from the questionable species of *Arachniotus, A. candidus, A. ruber* and *A. aureus,* by colonial and ascospore characters.

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THALASSIOMYCETES I. PRINCIPLES OF DELIMITATION OF THE MARINE MYCOTA WITH THE DESCRIPTION OF A NEW AQUATICALLY ADAPTED DEUTEROMYCETE GENUS [1, 2]

ROYALL T. MOORE [3] AND SAMUEL P. MEYERS

(WITH 1 FIGURE)

The last decade and a half has seen the recognition of an extensive mycota within the oceans that is represented mainly by the Ascomycetes and Deuteromycetes. The relatively small number of known marine fungi in these two classes is almost certainly a reflection of the infancy of the discipline and a lack of studies rather than of a dearth of organisms. The marine fungi of early reports were considered singular phenomena and the possibility of an indigenous fungal mycota was vigorously denied. Yet, considered objectively, the sea offers a most plausible ecological niche for fungi. Its temperature is not subject to great or sudden fluxes, the general light conditions are those associated with fungal habitats, and, particularly significant, the marine environment is rich in a vast and diverse array of nutritional substrates. Despite the evidence of a luxuriance of marine bacteria it was authoritatively held that the Eumycota generally could not be sufficiently salt-tolerant to be active and significant marine inhabitants. However, the prima-facie evidence presented by Barghoorn and Linder (1) has definitely controverted this objection.

Nonetheless, in recent literature the validity of marine fungi, as a concept, has again come to be questioned. Their ubiquitous presence is accepted but their halophilic metabolism is not. Unlike the great majority of plant and animals, many bacteria and fungi apparently are halinically indifferent and grow with nearly equal competence on distilled-water, sea-water, and even on hyper-sea-water media. As a

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result of this ability there has been introduced into the marine mycological literature such compromising terms as "'marine'" (*n.b.* inner quotes) and "so-called marine" [quasihalinic] (**6**, **7**) in reference to various taxa of marine-occurring fungi. This sort of hedging can only serve to introduce confusion into an already complex biological discipline. Fortunately, mere terminology and its convenient usage does not dictate the mode of activity of a particular organism in question nor its spectra of physiological adaptabilities.

The sea, unlike the land, where geographic isolation is the fundamental element of speciation, is a system of coincident isolating mechanisms (**8**). Salt concentrations vary from that of fresh water at stream mouths and ice masses to hypersalinity in poorly circulating coastal embayments; temperatures and substrates fluctuate not only from equator to poles, but, like light, with depth. These and other less prominent variables continuously interact to form a non-homogeneous environment in which all factors are subject to gradients. To calibrate one, *e.g.* salinity, and to apply it as a defining yardstick, while disregarding the others, fails to take realistically into account the sum total with which the sympatric populations of fungi must differentially integrate in order to be both successful and genetically specific in the marine environment (cf. **8**, **9**, **13**, **16**).

In the light of the above observations we consider it desirable that the complex of fungi circumscribed by the oceans be distinguished by the term *thalassiomycetes,* meaning simply "fungi of the sea," that is, fungal taxa of any class which are isolated predominantly or exclusively from marine habitats. Single recoveries of fungi must be carefully checked to ascertain that their presence in the sea is not merely fortuitous. The present methods of sampling (**11**), while effective, are such that it is quite probable that we are recovering the most common and possibly the least particular fungi. The development of more sophisticated isolation and culture techniques may be expected to reveal many fungi that exhibit fastidious requirements for growth. Genera such as *Torpedospora, Orbimyces,* and the new taxon, *Nia,* here described, appear to belong in this latter category. Though isolated but once from many thousands of panels examined, these fungi are valid thalassiomycetes. All three taxa produce spores uniquely adapted for aquatic dispersal. *Torpedospora* in its requirements is quite comparable to the ascomycetous genera cited below. Further, extensive examinations of freshwater habitats by a number of mycologists (**5**, **14**, **15**) have failed to disclose either these seemingly rare fungi or those that are ubiquitous in the sea. The ecological dichotomy between fungi associated with the

continental niches and the thalassiomycetes is too diametrically pronounced to be a sampling error. Before the mechanisms of this allopatrism can be explained, further and more intensive studies of the physiology of the organisms and their relationships to their respective environments will have to be completed.

In some lignicolous thalassic Ascomycetes salinity alone would appear to be a major limiting factor for growth and reproduction. Such genera as *Lulworthia, Ceriosporopsis, Antennospora, Peritrichospora, Lignincola, Arenariomyces,* and the above-mentioned *Torpedospora* produce sparse somatic growth in nutrient media prepared with distilled water and show no apparent initiation of ascogeny (**10**). Maturation of the fruiting structures of these fungi requires the presence of sea water itself or a well designed artificial sea water.

In contradistinction, most of the thalassic deuteromycetous species so far studied, including *Humicola alopallonella* Meyers & Moore, *Culcitalna achraspora* Meyers & Moore, *Piricauda arcticoceanorum* Moore, *Alternaria* sp. (F-109),[4] and *Stachybotrys atra* Corda, appear in culture to be facultative halophiles (**3**). (If the last named species is truly the same as Corda's original species and the terrestrial isolates identified with it, then it is one of an exceptionally few species native to both the continental and marine habitats (cf. **12**).) However, we have observed that several of our numbers isolated from the north-temperate and subarctic oceans are psychrophilic. Inoculates of these particular fungi showed little or no apparent growth after several weeks at room temperature (ca. 30–35° C), but when subsequently removed to a constant temperature of 25° these same cultures produced good to vigorous growth in less than a week.

It is erroneous to consider that somatic growth of a thalassiomycete on distilled-water media enriched with numerous metabolites places the organism in the category of a "fresh water strain" (**7**) of the parent type. Frequently marine-agar cultures become contaminated with airborne fungi (*e.g., Penicillium* spp.), and often terrestrial species inoculated onto such media exhibit good to vigorous growth. We would hardly consider the excellent development of airborne or terrestrial fungi on sea-water media to be evidence of salt-water strains of these particular species. These two antithetical reactions *merely* indicate that irrespective of the natural substrate the organisms can assimilate effectively the nutritional components of the particular medium. Since most standard commercial biological preparations, such as yeast-extract and peptone, contain various salts in addition to other components, perhaps

[4] Culture accession number of The Marine Laboratory.

it is not surprising that many marine species are capable of growing on enriched distilled-water media. As pointed out earlier by Vishniac (**17**), "The Fungi Imperfecti and Ascomycetes which have been isolated from sea water, bottom mud, or submerged wood . . . are adapted to growth in a broad range of osmotic pressure." Therefore, the present delimitation of the thalassiomycetes on the basis of ecology appears to be the only practical criterion at this time.

Nia gen. nov.

Sclerotium conidia in corticis coriacei superficie gignens, medulla gelatinosa. Conidia sphaerica vel ovalia, vibrissa una apicali, pluribus basalibus praedita.

Fructification sclerotial, producing conidia abundantly over the surface of the leathery cortex; center gelatinous. Conidia spherical to oval, with one apical and several basal vibrissa.

TYPE: *Nia vibrissa* Moore and Meyers.

(Etym.: *Ni*—Andean-Indian personification of the sea.)

Nia vibrissa sp. nov. FIG. 1

Fructificationes primum nitide aurantiaco-luteae, serius sature aurantiacae, denique nonnumquam aurantiaco-brunneae, globosae, ad aliquot mm diam. Conidia sessilia, continua, ovalia vel suboblonga, specie granulosa, (7.5–)10–15 × 6.5–8.5 (–10) μ, vibrissam unam terminalem 25–40 μ long., basales (2–)3–4, 25–30 μ long., omnes fastigatas, rectas vel parum fluexuosas, hyalinas praedita.

In ligno putrido *Fagi sylvaticae*, in mare immerso, Biscayne Bay, Florida, et preparationes microscopicae in Herb. FH, HOLOTYPUS; cultura viva *S. P. Meyers F-183* et preparatio microscopica *RTM I:261*, Isotypi.

Fructifications initially a shiny orange-yellow, becoming deep orange, and, finally, in some instances, orange-brown, globose, large, up to several millimeters in diameter. Conidia sessile, amerosporous, oval to slightly oblong, appearing granular, (7.5–)10–15 × 6.5–8.5(–10) μ, bearing a single terminal bristle 25–40 μ long, and (2–)3–4 basal bristles 25–30 μ long, bristles tapering, straight to slightly flexuous, hyaline.

(Etym.: *vibrissa*—whisker, bristle, in reference to the conidial appendages.)

The fungus was isolated initially on beech wood shavings that had been submerged for several months in Biscayne Bay, Florida. The large, orange, globose fruiting structures appeared on the wood after incubation for two to three weeks in a sealed petri dish in the laboratory. *Nia vibrissa* grows well in artificial culture on a sea-water medium of 1.0% glucose and 0.1% yeast-extract (Difco). Often large, irregular,

light-colored to orange stroma-like sterile structures are produced on the surface of the agar. Numerous irregular gelatinous masses with no apparent organization also are produced on the agar surface. These gelatinous masses are unusual in that they often develop in a ring around the area of inoculation. However, in the first subculture from the original isolation the typical globose structures developed in which were produced the characteristic appendaged spores. In shake cultures of 0.1% yeast-extract-sea-water broth, fimbriate, translucent, light-colored, globose structures are produced that, when held against the light, are seen to contain a small dense core.

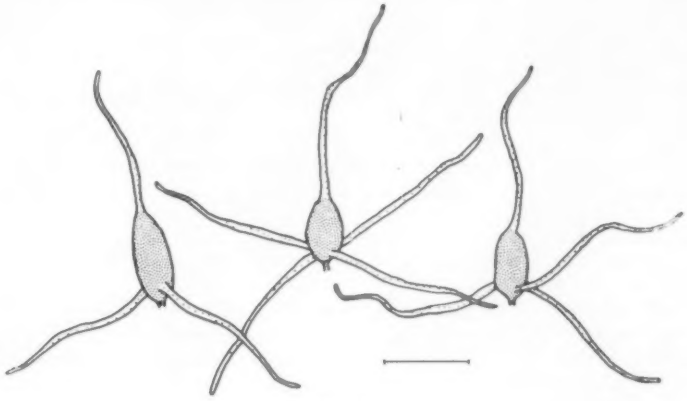

FIG. 1.

Nia vibrissa, typical conidia showing the single terminal and several basal processes. Scale line equals 15 μ.

Nia vibrissa is a unique thalassiomycete from several aspects. Its sclerotium-like fruiting structure makes it problematical whether the fungus belongs in the Tuberculariaceae or in the Melanconiaceae. In the keys of Clements and Shear (2) it would appear to be close to *Eriosporella* in the former family and *Illosporium* in the latter. In either family, though, it represents the first report of a marine representative. *N. vibrissa* also is exceptional in its production of conidia that are adapted to an aquatic habitat. Of known marine fungi such adaptations are common in the Ascomycetes, but *Orbimyces* and an undescribed species with tetraradiate conidia represent the only other thalassic Deuteromycetes with such adaptations.

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ON THE BIOSYNTHESIS OF GIBBERELLINS FROM CARBON-14-SUBSTRATES BY FUSARIUM MONILIFORME[1]

GUNTER ZWEIG AND J. E. DEVAY

The biosynthesis of C^{14}-labeled gibberellic acid was first accomplished by using uniformly labeled sucrose-C^{14} as substrate for *Fusarium moniliforme* (Watanabe, **1957**). Others have studied the incorporation of acetate-1-C^{14}, acetate-2-C^{14} and mevalonic acid-2-C^{14} into gibberellic acid by the same fungus (Birch *et al.*, **1958**; Zweig *et al.*, **1958**; Zweig and DeVay, **1959**). These studies have led to the hypothesis that gibberellic acid was synthesized by the loss of four carboxyl groups and one angular methyl group per four molecules of mevalonic acid. Recently, 3-methyl crotonic acid has been suggested as an intermediate in the biosynthesis of gibberellic acid (Redemann and Meuli, **1959**).

The present study was undertaken to elucidate the biosynthetic pathway of gibberellic acid by *F. moniliforme* by growing the microorganism on media containing various carbon-14 substrates. These studies have also led to a practical method of synthesizing randomly labeled gibberellic acid-C^{14}.

MATERIALS AND METHODS

Materials: Acetate-1-C^{14} and acetate-2-C^{14} were purchased from Research Specialties Co., Richmond, California and mevalonic acid-2-C^{14} from Tracerlab, Inc., Waltham, Massachusetts. Uniformly labeled sucrose-C^{14}, glucose-C^{14}, and fructose-C^{14} were obtained from Dr. Richard D. Durbin,[2] Dept. of Biochemistry, University of California, Berkeley. 3-Methyl crotonic acid-3-C^{14} and 3-methyl crotonic acid-3-methyl-C^{14}, 4-C^{14} were obtained from Isotope Specialties Co., Burbank, California. Gibberellic acid (A_3) and gibberellin A_1 were generously donated by Abbott Laboratories, North Chicago, Illinois.

[1] Presented in part at 1958 meeting of the American Society of Plant Physiologists, Bloomington, Indiana and IX International Botanical Congress, Montreal, 1959.

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Cultural Conditions: Cultures of *Fusarium moniliforme* Sheld., strain NRRL 2284, were grown in 250-ml Erlenmeyer flasks containing the following synthetic medium: Sucrose, 20 g; $MgSO_4 \cdot 7H_2O$, 3 g; NH_4Cl, 3 g; KH_2PO_4, 3 g; $FeCl_3 \cdot 6H_2O$, 0.24 mg; $ZnCl_2$, 0.15 mg; H_3BO_3, 0.06 mg; $CuCl_2 \cdot 2H_2O$, 0.05 mg; $MnCl_2 \cdot 4H_20$, 0.04 mg; $NaMoO_4 \cdot 2H_2O$, 0.03 mg; distilled water to 1 liter.

The portion of the medium containing all of the inorganic components was autoclaved for 20 minutes at 121° C. An aqueous solution of sucrose of the appropriate strength, sterilized by passage through a Seitz filter, was pipetted aseptically into the culture flasks. Flasks containing 100 ml of the above medium were seeded with mycelium from agar slants and agitated on a New Brunswick rotary shaker (214 rpm) for 72 hours at 24° C. Each culture flask was capped with a disc of Whatman No. 2 filter paper held in place with a rubber band.

The experimental flasks were then seeded with 25 ml aliquots of the combined seed cultures to give a total volume of 100 ml of medium in each flask. Maximum growth of the organism, as measured by dry weight, was reached in approximately 72 hours. After an additional 48 to 72 hours, the cultures were producing 50–100 μg of gibberellic acid per ml of culture medium, as determined by the dwarf maize (d-1 mutant) bioassay (Neely and Phinney, **1957**; Zweig and Cosens, **1959**). Sugars in the culture filtrates were analyzed (Dubois *et al.*, **1956**) at the end of the incubation period (144 hours), and no sucrose but only a trace of glucose was detected. Fructose was present in concentrations ranging from 5 to 10 mg/ml.

At this stage of development, subsamples of the cultures were used for the radioactive experiments. The fungus grew as mycelial fragments in the shake cultures, and the cellular suspensions were easily pipetted. In preliminary studies on the conversion of radioactive substrates to gibberellic acid, replacement cultures were employed by suspending washed cells in the synthetic medium devoid of sugar and nitrogen. Gibberellic acid production, however, was unsatisfactory under these conditions, and in subsequent studies the original culture medium was used.

A pint-size Mason jar fitted with a screw cap was found to be a suitable incubation chamber for the radioactive experiments. Five ml of N NaOH was placed in the bottom to absorb any evolved carbon dioxide. Five ml of the mycelial suspension was pipetted into 30 ml beakers which were secured to the walls of the jars with plastic putty. One ml amounts of sterile aqueous solutions containing C^{14}-labeled substrates were added to the beakers, and the cultures were incubated at

28° C for 48 to 72 hours without agitation under ordinary light conditions in the laboratory. Cultures kept in darkness usually produced less gibberellic acid than cultures which were kept in the light.

Isolation of Gibberellic Acid-C^{14}: At the end of the incubation period, a weighed amount of pure gibberellic acid, dissolved in 1.0 ml of absolute ethanol, was added to each culture. The mycelial suspension was filtered with Whatman No. 1 paper, and the gibberellic acid was isolated by a modification of published methods (Borrow *et al.*, **1955**; Stodola *et al.*, **1955**): The filtrates (pH 1.8–2.0) were agitated with 100 mg of Darco G-60 charcoal on a Burton shaker for 20 minutes. The charcoal was filtered, partially dried, and eluted with several 100 ml portions of acetone. The combined acetone extracts were dried *in vacuo*, and the residue was dissolved in 0.2 ml ethanol and 2.0 ml ethyl acetate. Upon the addition of petroleum ether (b.p. 30–65° C), a white flocculent material precipitated. This was recrystallized repeatedly from ethyl acetate-petroleum ether and was air-dried.

Identification of Gibberellic Acid: Recrystallized gibberellic acid-C^{14} was examined by paper chromatography and paper electrophoresis by the methods described below. The paper chromatograms and electrophoresis strips were scanned for radioactivity with a Forro gas flow chromatogram scanner. For more discerning examination, the paper strips were placed on Kodak non-screen X-ray film for periods up to 4 weeks.

Paper Chromatography (Mitchell, **1958**): Whatman No. 1 paper and the ascending technique were used (Block *et al.*, **1958**). The solvent was *tert*-amyl alcohol:acetone:*n*-butanol:ammonium hydroxide:water = 25:25:25:10:15 v/v. The chromogenic solution was prepared by mixing 4 volumes of 1 per cent aqueous potassium periodate and 1 volume of 1 per cent potassium permanganate in 1 per cent sulfuric acid. (This solution was stable for months when kept under refrigeration.)

Gibberellic acid was visualized by dipping the developed chromatogram into the chromogenic solution and immediately washing the paper in cold running tap water. Brown spots on a white background resulted. Reducing sugars (R_f 0.12) and unknown excretory products from *F. moniliforme* (R_f 1.0) gave similar brown spots with this reagent, but their respective R_f values were significantly different from that of the gibberellins (see TABLE I). R_f values for mevalonic acid, sucrose, glucose, fructose, and acetate were determined from the autoradiograms of these substrates (see TABLE I).

Paper Electrophoresis (Block *et al.*, **1958**): For paper electrophoresis, a Spinco apparatus with barbital buffer (pH 8.6; $\mu = 0.05$) at 300

V DC was used. The paper strips, after electrophoresis (2 hours run), were dipped into the same chromogenic solution as used for paper chromatograms. The electrophoretic mobility of gibberellic acid was + 2.5 cm/hour, corrected for endosmosis with glucose. No interference from other substances was encountered using this buffer system.

Coincidence of the exposed area on the autoradiogram with the brown spot on the paper chromatograms, due to known gibberellic acid as a marker, was taken as positive identification of C^{14}-labeled gibberellic acid.

TABLE I

R_f VALUES; PAPER CHROMATOGRAPHY *

Mevalonic acid	0.12
Sucrose	0.12
Glucose	0.12
Acetate	0.12
Fructose	0.12
Gibberellic acid (A_3)	0.28
Gibberellin A_1	0.28
Mevalonic acid metabolite	0.46

* Experimental conditions described in text.

Determination of Radioactivity: Aliquot samples, plated on 1-inch diameter planchets, were dried and counted, using a thin-end window G.-M. tube or gas-flow counter. No corrections were made for self-absorption. Acetate-C^{14} samples were adjusted to pH 9 with 0.1 N NaOH prior to sampling.

RESULTS AND DISCUSSION

Experiments with C^{14}-Labeled Acetate (Qualitative): Autoradiograms of the crude culture medium, chromatographed directly before the isolation of C^{14}-labeled gibberellic acid, showed that acetate-1-C^{14}, and acetate-2-C^{14} were metabolized by *F. moniliforme* into at least 8 other carbon compounds, besides gibberellic acid. Some of these compounds might represent the precursors of gibberellic acid. No significant difference was observed qualitatively in the labeling patterns from acetate-1-C^{14} and acetate-2-C^{14}. Autoradiograms of the isolated C^{14}-labeled gibberellic acid showed that the major radioactive component with an R_f of 0.28 was identical with that of carrier-gibberellic acid. Autoradiograms of the purified C^{14}-labeled gibberellic acid showed 3 additional minor unknown components at R_f 0, 0.12 and 0.46. The 0.12 component was shown to be acetate, and the 0.46 spot had the same mobility as the mevalonic acid metabolite, discussed below.

TABLE II

CARBON DIOXIDE EVOLUTION FROM C^{14}-LABELED ACETATE BY F. MONILIFORME

	Acetate-1-C^{14}	Acetate-2-C^{14}
Experiment 1	69.7*	46.8*
Experiment 2	26.1	18.4
Experiment 3	23.9	8.3
Experiment 4	54.2	39.5

* Per cent of the radioactivity originally added to the subculture. Average of analyses of duplicate cultures.

Experiments with Acetate-C^{14} (Quantitative): Results from the oxidation of acetate by *F. moniliforme* in terms of $C^{14}O_2$ evolution are summarized in TABLE II. In each experiment the rate of oxidation of the carboxyl group was higher than that of the methyl group. This differential rate of oxidation would be predicted if the reactions proceeded via the Krebs cycle (Haurowitz, **1955**).

The specific activity of the isolated C^{14}-labeled gibberellic acid from each experiment, performed in duplicate, was calculated, but no allowance was made for the 3 minor radioactive components described above. From the specific activities and the weight of the carrier-gibberellic acid added, the total radioactivity of the biosynthesized gibberellic acid was calculated. It was assumed that the weight of biosynthesized gibberellic acid was insignificant, and that in each replicate flask equal amounts of

TABLE III

CONVERSION OF ACETATE-1-C^{14} AND ACETATE-2-C^{14} INTO C^{14}-LABELED GIBBERELLIC ACID (EXPERIMENT 3)

	Added radioactivity, CPM	CO_2, CPM	$\%CO_2$	Available radioactivity, CPM	Gibberellic acid		% Carbon converted based on radioactivity:	
					Specific activity, CPM/mg.	Total radioactivity,* CPM	Initial	Available
Acetate-1-C^{14}	7,809,000	1,825,500	23.4	5,983,500	542	13,550	0.17	0.23
Acetate-1-C^{14}	7,809,000	1,900,500	24.3	5,908,500	473	11,825	0.15	0.20
Acetate-2-C^{14}	2,938,000	262,600	8.9	2,675,400	328	8,200	0.28	0.31
Acetate-2-C^{14}	2,938,000	223,000	7.6	2,715,000	275	6,875	0.23	0.25

	Initial radioactivity	Available radioactivity
Conversion efficiency: $\frac{\text{Acetate-2-}C^{14}}{\text{Acetate-1-}C^{14}}$	1.59	1.30

* 25.0 mg of carrier GA was added.

gibberellic acid had been produced. The isolation yield did not affect these calculations since they were based on the isotope dilution method (Aronoff, **1956**).

The per cent carbon assimilated from acetate into gibberellic acid was calculated on the basis of "initially added radioactivity" and "available radioactivity." "Available radioactivity" was the initial radioactivity corrected for loss due to CO_2 evolution. TABLE III is an illustration (Experiment 3) of such an experiment and the subsequent calculations. It may be seen from this experiment that the conversion efficiency from the methyl-carbon to gibberellic acid was significantly greater than that of the carboxyl-carbon. The same observation was made in three other

TABLE IV

CARBON EFFICIENCY RATIOS FOR C^{14}-LABELED ACETATE CONVERSION TO C^{14}-LABELED GIBBERELLIC ACID

Experiment No.	$\frac{\text{Acetate-2-C}^{14}}{\text{Acetate-1-C}^{14}}$	
	Based on initial radioactivity	Based on available radioactivity
1	2.49	1.88
2	2.03	1.85
3	1.59	1.30
4	2.26	1.69

experiments, the results of which are summarized in TABLE IV. The experimental efficiency ratios for acetate-2-C^{14} and acetate-1-C^{14} based on "available radioactivity" were found to be between 1 and 2 which would tend to support the theory of a biosynthetic pathway involving mevalonic acid as an intermediate (Birch *et al.*, **1958**). The theoretical efficiency based on this theory is 1.375 according to the following reactions: 12 acetate → 4 mevalonic acid → gibberellic acid + 5 carbon dioxide. The CO_2 was the result of decarboxylation and loss of an angular methyl group from mevalonic acid. Subsequent experiments reported below indicate that mevalonic acid may not be directly involved in the biosynthesis of gibberellic acid.

Experiments with Mevalonic Acid-C^{14} and 3-Methyl Crotonic Acid-C^{14}: In order to test the biosynthetic pathway of gibberellic acid via mevalonic acid or 3-methyl crotonic acid, cultures of *F. moniliforme* were incubated with these substrates labeled in the following positions: mevalonic acid-2-C^{14}, 3-methyl crotonic acid-3-C^{14}, and 3-methyl crotonic acid-3-methyl-C^{14}, 4-C^{14}. Starting with mevalonic acid-2-C^{14}, a

radioactive product was isolated, having the same solubility and charcoal adsorption characteristics as gibberellic acid but an R_f value of 0.46 (see TABLE I). As mentioned before, a minor unknown radioactive component resulting from the metabolism of acetate had the same R_f value as the mevalonic acid metabolite. These substances, therefore, may be identical and may represent a precursor of gibberellic acid in cultures containing either acetate or mevalonic acid as the radioactive substrate. Gibberellins A_1 and A_3 have an R_f value of 0.28 in the solvent tested, and it was ruled out that this spot was due to other known

TABLE V

CARBON DIOXIDE EVOLUTION FROM C^{14}-LABELED SUBSTRATES BY F. MONILIFORME

	Starting radioactivity, CPM	$C^{14}O_2$, CPM	% C^{14} converted to $C^{14}O_2$
(Experiment 2)			
Mevalonic acid-2-C^{14}	2,520,000	29,825	1.18
Sucrose-C^{14}	185,350	27,690	14.94
Glucose-C^{14}	12,260,000	47,865	0.39*
Fructose-C^{14}	5,075,000	35,135	0.69
(Experiment 4)			
Glucose-C^{14}	348,500	70,250	20.16*
Mevalonic acid-2-C^{14}	312,300	13,900	4.45
3-Methyl crotonic acid-3-methyl-C^{14}, 4-C^{14}	387,200	81,550	21.06
3-Methyl crotonic acid-3-C^{14}	273,600	87,750	32.07

* In Experiment 2 cells were washed and suspended in the replacement medium, whereas in Experiment 4 the cells were incubated in the original culture medium. This difference in cultural conditions may account for the higher $C^{14}O_2$ production from glucose-C^{14} in Experiment 4.

gibberellins.[3] Due to the low concentrations of this metabolite, its biological assay with dwarf maize was not possible. Carbon dioxide evolution in a 72-hour period amounted to 4.5 per cent of starting radioactivity with mevalonic acid as substrate, demonstrating that mevalonic acid actually was metabolized.

When 3-methyl crotonic acid (senecioic acid) was used as a substrate, the conversion efficiency of radioactive carbons 3 and 4 to gib-

[3] A recently isolated gibberellin-C^{14} in our laboratories from *Fusarium moniliforme* may be a newly discovered fungal gibberellin. Its infrared spectrum exhibited the absence of a strong 5.7 micron band (acid or lactone) and the presence of an absorption band at 6.15 micron (so far unassigned).

berellic acid seemed to be of the same order (2.04 vs. 2.23 per cent, based on available radioactivity) indicating that 3-methyl crotonic acid was converted *in toto* to gibberellic acid. Carbon dioxide production in 72 hours was 32.1 per cent and 21.1 per cent respectively. These experiments strongly suggested that, using the strain of *F. moniliforme*, NRRL 2284, under the stated experimental conditions, 3-methyl crotonic acid rather than mevalonic acid was converted to gibberellic acid. Pure C^{14}-labeled gibberellic acid has been previously isolated from 3-methyl crotonic-3-C^{14} (Redemann and Meuli, **1959**).

TABLE VI

SUMMARY OF CARBON YIELDS FOR THE REACTION: C^{14}-LABELED SUBSTRATE → GIBBERELLIC ACID-C^{14}

Exp.**	C^{14}-labeled substrate	Radioactivity (CPM)		
		Added	GA isolated	% C^{14} Yield
1	Acetate-1-C^{14}	2,180,000	16,293	0.81
1	Acetate-2-C^{14}	1,755,000	35,466	2.02
2	Acetate-1-C^{14}	7,809,000	70,802	0.91
2	Acetate-2-C^{14}	5,876,000	108,733	1.85
2	Mevalonic acid-2-C^{14}	2,520,000	trace	0
2	Sucrose-C^{14}	185,350	trace	0
2	Glucose-C^{14}	12,260,000	trace	0
2	Fructose-C^{14}	5,075,000	trace	0
4	Glucose-C^{14}	348,500	6,062*	1.74*
4	Mevalonic acid-2-C^{14}	312,300	738*	0.24*
4	Acetate-1-C^{14}	5,215,700	16,365	0.31
4	Acetate-2-C^{14}	1,572,100	10,994	0.70
4	3-Methyl crotonic acid-3-C^{14}	273,600	3,800	1.39
4	3-Methyl crotonic acid-3-methyl-C^{14}, 4-C^{14}	387,200	6,842	1.76

* Radioactivity due to impurity.
** Experiments 1 and 2: replacement media; Experiment 4: original medium.

Experiments with C^{14}-Sugars: The following additional uniformly C^{14}-labeled compounds were added to the fungus cultures: sucrose (3.0 μc), D-glucose (198 μc), and D-fructose (82 μc). After 48 hours incubation, attempts were made to isolate C^{14}-labeled gibberellic acid with the addition of a weighed amount of nonlabeled material. No significant amounts of radioactive gibberellic acid were isolated from any of the C^{14}-labeled sugars. $C^{14}O_2$ production varied considerably for different substrates and experiments (TABLES II, V), but high $C^{14}O_2$ values usually were associated with relatively high C^{14}-gibberellic acid yields. These experiments showed that at low sugar concentration no significant amounts of gibberellic acid were produced. Others (Borrow *et al.*, **1955**; Stodola *et al.*, **1955**) have reported the production of gib-

berellic acid from high concentrations of sugars. Our results indicated that the addition of sugars resulted in low carbon conversion to gibberellic acid.

Comparison of C^{14}-Substrates: A study of carbon yields of isolated C^{14}-labeled gibberellic acid revealed that C^{14}-labeled acetate and 3-methyl crotonic acid, but none of the other C^{14}-labeled compounds, were converted to C^{14}-labeled gibberellic acid in significant yields. These results are summarized in TABLE VI. Although it appeared that C^{14}-labeled glucose and mevalonic acid resulted in significant amounts of C^{14}-labeled gibberellic acid, an examination of the autoradiograms showed that this radioactivity was due to starting material. In the case of 3-methyl crotonic acid an additional metabolite was produced, which survived repeated recrystallization of isolated gibberellic acid. This suggested the similarity of the metabolite of 3-methyl crotonic acid with gibberellic acid. It appeared that the only successful conversion of C^{14}-labeled substrates to C^{14}-labeled gibberellic acid occurred with acetate-1-C^{14}, acetate-2-C^{14}, 3-methyl crotonic acid-3-C^{14}, and 3-methyl crotonic acid-3-methyl-C^{14}, 4-C^{14}. Sucrose, glucose, and fructose were excellent carbon sources for *F. moniliforme* as measured by mycelial dry weight. However, acetate in low concentration (0.04 M) was a poor carbon source and in higher concentrations (0.17M) was inhibitory.

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SUMMARY

Conditions are described under which C^{14}-labeled gibberellic acid was synthesized by cultures of *Fusarium moniliforme* (NRRL 2284) from acetate-1-C^{14} and acetate-2-C^{14}. The results showed that the methyl-carbon of acetate was converted more efficiently to gibberellic acid than the carboxyl-carbon. Also, 3-methyl crotonic acid-3-C^{14} and 3-methyl crotonic acid-3-methyl-C^{14}, 4-C^{14} were converted into small quantities of C^{14}-labeled gibberellic acid. Other substrates which were studied included mevalonic acid-2-C^{14}, uniformly C^{14}-labeled sucrose, glucose, and fructose. These experiments confirmed that acetate and

3-methyl crotonic acid may be precursors in the biosynthesis of gibberellic acid by *F. moniliforme*.

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MICROBIOLOGICAL PRODUCTION OF CAROTENOIDS. VI. SOME FACTORS AFFECTING SPORULATION AND GROWTH IN THE CHOANEPHORACEAE

C. W. Hesseltine and C. R. Benjamin

(with 6 figures)

During the course of studies made in our laboratory on the submerged-fermentation production of carotenoids, reported by Hesseltine and Anderson (**1957**, **1958**), Anderson et al. (**1957**, **1958**), and Ciegler et al. (**1959a**, **1959b**), the problem of maintaining consistent, high yields was encountered. This problem stimulated the initiation of a series of experiments on the sporulation and growth of the fungi of the family Choanephoraceae, toward the end of producing inocula of (+) and (−) strains which would contain equivalent numbers of spores and equal amounts of mycelia. This series of experiments is the subject of the present report.

As early as 1879, Cunningham noted that strains of *Choanephora infundibulifera* (Currey) Sacc. lost their ability to form conidia after three successive transfers on culture media. On the other hand, Wolf (**1917**) reported that *C. cucurbitarum* (Berk. & Rav.) Thaxter could be grown for 15 generations with no resultant decrease in asexual reproduction. Cunningham also reported that sporangia were not seen on the host but that they were produced on colonies growing on exhausted media. The same investigator (**1895**) reported that the main factors influencing fruiting of *C. simsoni* Cunn. were nutritional ones. Thus, when nutrients were present in large amounts, no fruiting occurred; with lesser amounts, conidia were produced; and if the nutrients were very poor, only sporangia were formed. Cunningham (**1879**), as well as Möller (**1901**), Thaxter (**1903**), Palm and Jochems (**1924**) and others, reported that conidia in the Choanephoraceae were formed at night and matured early in the morning. Thaxter (**1903**) found that *C. cucurbitarum* produced few fructifications when grown in a saturated atmosphere and that it seldom fruited in test-tube cultures. Saito and Naganishi (**1915**), studying *Cunninghamella mandshurica* (*Choanephora*

cucurbitarum) on rice, noted that the optimum temperature for growth was about 25–30° C, but that growth occurred from 15–36° C. Their strain liberated diastase, lipase, protease and rennet but could not break down trehalose, sucrose or lactose. Couch (**1925**), studying *C. conjuncta* Couch, reported that good conidial production could be obtained if corn-meal filtrate was added to the agar. He also noted that if a high amount of agar (14 g/l) were used in the medium, better sporulation occurred than if the agar content were lower (10 g/l). Couch found that if cultures were kept in total darkness, no conidia were formed.

Miyake and Ito (**1935**) reported that *C. cucurbitarum* (described by them as *Choanephoroidea cucurbitae*) formed conidia within 30 hours at 20° C and that at 55 hours formed them at 10–25° C. Vegetative growth was greatest at 30° C if colony diameters were used as a measure of growth.

Goldring (**1936**) studied two strains of *Blakeslea trispora* Thaxter, using a potato medium, and found that the quantity of nutrients affected sporangial formation in one strain. In this strain media poor in nutrients favored sporangial development and those rich in nutrients favored sporangiole formation. No growth occurred at 6° C or 40° C and at 37° C only mycelium was formed. The strains grew best under slightly acid conditions, although growth occurred even at pH 9.6.

Christenberry (**1938**) showed that light did not affect vegetative growth of *C. cucurbitarum*, but that alternating light and dark periods were best for optimal fruiting.

In a study of two *Choanephora* spp. strains, Kishore (**1940**) demonstrated that sporangia and conidia were formed at 25° C and 30° C, but none were formed at 33° C. A relative humidity of 60% was much better than one of 95% for sporangial and conidial formation in these strains.

Barnett and Lilly (**1950**) found that spore inoculum from *C. cucurbitarum* could be preserved by suspending the spores in sterile distilled water and placing them in a freezing compartment of a refrigerator. When inoculum was needed, the ice was melted slowly. Spore stocks stored in this fashion remained in a viable condition for 11 months. Their strains had a requirement for thiamine. For optimum sporulation the thiamine was high (25 μg/l) while the glucose was low (2 g/l). Asparagine and casein hydrolysate were excellent nitrogen sources and glucose was the best carbohydrate. Good aeration of the cultures was necessary for conidial formation. Optimum temperature for conidial production was near 25° C, although excellent mycelial growth occurred

at 34° C. A temperature of 31° C was unfavorable for conidial formation but allowed the formation of many large sporangia. In a later study Lilly and Barnett (**1953**), using the same species, studied its utilization of sugars. It was found that *C. cucurbitarum* could use glucose, d-fructose, mannose, d-galactose, l-arabinose, d-xylose, maltose and cellobiose, but did not utilize l-sorbose, sucrose, lactose and raffinose. These investigators (**1955**) stated than an accumulation of carbon dioxide in closed vessels reduced sporulation. High relative humidities likewise reduced conidial formation but stimulated sporangial fruiting at 25° C. Nitrogen utilization studies by the same authors (**1956**) showed that *C. cucurbitarum* could use ammonium salts, urea, l-asparagine, dl-alanine, l-arginine, l-glutamic acid and l-proline. Glycine was not utilized and growth in media containing l-leucine and dl-phenylalanine was very poor. Lilly and Barnett (**1956**) found that *C. cucurbitarum* utilized casein hydrolysate as the sole source of carbon but not glycine or arginine. Kawakami (**1955**) noted that glutamic acid was excellent for growth and sexual reproduction of *Blakeslea* and *Choanephora* and that urea and asparagine were next best. Ammonium sulfate was unsatisfactory unless $CaCO_3$ was added to the culture medium. Thiamine was required for growth by *C. cucurbitarum, C. infundibulifera* and *B. trispora* according to Kawakami and Nehira (**1956**).

A *Penicillium* strain promoted sporulation of *B. trispora* according to Weston (**1951**).

MATERIALS AND METHODS

The cultures used throughout this investigation were: *Blakeslea trispora* NRRL 2456(+) and NRRL 2457(−); *Choanephora cucurbitarum* NRRL 2744(+) and NRRL 2745(−); *C. circinans* NRRL 2546(+) and NRRL 2548(−); and *C. conjuncta* NRRL 2560(+) and NRRL 2561(−). Stock cultures were grown either in tubes or Petri dishes and the resulting mycelium was used as inoculum. The potato dextrose agar (PDA) used was prepared by the method of Haynes et al. (**1955**) and the synthetic mucor agar (SMA) was prepared as described by Hesseltine (**1954**).

A basal medium of the following composition was used for studies on the utilization of nitrogen and carbon: glucose 2 g or asparagine 1 g,[1] K_2HPO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, Fe^{+++} 0.2 mg, Zn^{++} 0.2 mg, Mn^{++} 0.2 mg, thiamine 12.5 μg, agar 20 g and distilled water 1000 ml.

[1] Glucose (2 g/l) was added to media containing the various nitrogen compounds and asparagine (1 g/l) served as the nitrogen source for the carbon utilization studies.

A modification of the basal preparation, designated as Medium 5, consisted of: maltose 1.0%, $(NH_4)_2SO_4$ 0.1%, $MgSO_4$ 0.02%, $CaCO_3$ 0.02%, thiamine 12.5 μg/l, $FeSO_4$ 0.001%, KCl 0.05%, K_2HPO_4 0.05% and agar 1.5%.

EXPERIMENTS AND RESULTS

For the first experiment each of the eight strains was grown on PDA slants and inoculated onto the above basal medium to which 1 g/l of the various nitrogen sources listed in TABLE I had been added. The plates were run in duplicate at 25° C and the experiment was repeated. The plates were examined at 4 to 5 days and in no case was fruiting on the

TABLE I

EFFECT OF VARIOUS NITROGEN SOURCES ON GROWTH AND SPORULATION OF CHOANEPHORACEAE ON SOLID MEDIA*

Nitrogen sources	*B. trispora*				*C. cucurbitarum*				*C. circinans*				*C. conjuncta*			
	NRRL 2456		NRRL 2457		NRRL 2744		NRRL 2745		NRRL 2546		NRRL 2548		NRRL 2560		NRRL 2561	
	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S
N-free	−	2	−	2	−	1–2	−	1–2	−	2	−	2	−	2–3	−	2
$(NH_4)_2SO_4$	+	3	+	3	+	3	+	1–2	+	2–3	+	1–2	+	2–3	+	3
$NaNO_2$	−	0	−	0	−	0	−	0	−	0–1	−	0	−	0	−	0
KNO_3	−	2	−	2	−	2	−	2	−	2	−	1–2	−	2–3	−	2
Urea	+	2–3	+	2–3	+	3	+	2	+	0–2	+	0–2	+	2	+	0–2
Asparagine	+	3	+	3	+	3	+	2	+	1–2	+	0	+	3	+	3

* Key: G = growth. S = Sporulation. + = Growth exceeding that of N-free control. − = Growth not exceeding that of N-free control. 0 = No sporulation. 1 = 1–10 fruiting heads. 2 = 10–100 fruiting heads. 3 = 100 or more fruiting heads.

single small block of inoculum included in the count. The results are summarized in TABLE I. The rating numbers in TABLES I, II and III are recorded per plate. When two numbers are given this indicates the range of counts from replicate experiments.

As anticipated, growth occurred on the N-free control plates because of the residual nitrogen carried over in the inoculum, and the mycelium actually reached the edges of the dishes. The growth on all other plates was compared to that of the controls. It is obvious that ammonium sulfate, urea and asparagine were utilized by all species of the Choanephoraceae tested whereas neither $NaNO_2$ nor KNO_3 was utilized. With the possible exception of *C. cucurbitarum*, $NaNO_2$ actually was inhibitory to growth and sporulation as compared with the controls. Both ammonium

sulfate and asparagine were adjudged to be superior to urea for sporulation and growth since the mycelial growth of all strains on the urea medium had a water-soaked appearance. *C. conjuncta* produced only conidiophores and *C. circinans* only sporangia, the latter of which were larger and more normal on ammonium sulfate. With *C. cucurbitarum*, conidiophores and sporangiophores were seen on all media supporting growth, although very few sporangia were present on urea. *B. trispora* formed both sporangia and sporangioles on all media except urea, on which only sporangioles were formed.

TABLE II

EFFECT OF VARIOUS CARBON SOURCES ON GROWTH AND SPORULATION OF CHOANEPHORACEAE*

Carbon sources	*B. trispora*				*C. cucurbitarum*				*C. circinans*				*C. conjuncta*			
	NRRL 2456		NRRL 2457		NRRL 2744		NRRL 2745		NRRL 2546		NRRL 2548		NRRL 2560		NRRL 2561	
	G	S	G	S	G	S	G	S	G	S	G	S	G	S	G	S
C-free	−	3	−	3	−	3	−	1–2	−	2	−	2	−	2	−	3
i-erythritol	−	3	−	3	−	2	−	0–1	−	3	−	2	−	1–2	−	3
glycerol	+	3	+	3	+	3	+	1–2	+	1–2	+	2	−	1	+	2
d-xylose	−	2	−	1–2	+	2–3	+	1	−	3	+	2	−	0	−	2
l-arabinose	+sl	3	+	3	+	2–3	+sl	2	+sl	3	+sl	3	+sl	2	+	3
d-arabinose	−	2	−	3	−	2	−	1	−	3	−	2	−	0	−	2
d-ribose	−	2–3	−	3	−	1–2	−	1–2	−	[2]	−	[2]	−	1	+sl	2
glucose	+	[3]	+	3	+	[3]	+	2	+	[3]	+	[1–2]	+	[3]	+	2–3
galactose	+	[3]	+	3	+	3	+	2	+	2	+	1	+	3	+	[3]
l-sorbose	−	2	−	3	−	0–2	−	0	−	1–2	−	0	−	0	−	2
trehalose	+	3	+	3	+	3	+	1	+	2	+	1	+	3	+	3
melibiose	−	3	−	3	−	3	−	1	−	2–3	−	2	−	2	−	3
lactose	−	3	−	3	−	3	−	2	−	2	−	2	−	1–2	−	3
sucrose	−	3	−	3	−	3	−	2	−	2–3	−	2	−	3	−	3
cellobiose	+	[3]	+	[3]	+	[3]	+	[3]	+	2	+	1	+	[3]	+	[3]
maltose	+	3	+	[3]	+	3	+	1–2	+	2–3	+	1–2	+	2–3	+	[3]
raffinose	−	3	−	3	−	3	−	1	−	2–3	−	2	−	2	−	3
soluble starch	−	2–3	+	3	+	2–3	+	1	+	2	+sl	2	−	1	+	2

* Key: G = Growth. S = Sporulation. + = Growth exceeding that of C-free control. − = Growth not exceeding that of C-free control. 0 = No sporulation. 1 = 1–10 fruiting heads. 2 = 10–100 fruiting heads. 3 = 100 or more fruiting heads. sl = Slight. [ ] = Best sporulation.

A second series of experiments was performed to determine growth and sporulation on each of 17 different carbon sources. The basal medium was supplemented with 1 g of asparagine and 2 g of each carbon compound per liter. Incubation was at 25° C and the inoculum was handled as before. The results are summarized in TABLE II.

With d-xylose there was a pronounced delay in the growth of those strains rated negative for this sugar, but later examination showed that all strains eventually grew better than the control. The *C. cucurbitarum* isolates were positive for this sugar from the beginning, indicating that they used the material without a period of adaptation. From TABLE II it can be noted that carbohydrates giving the best sporulation do not

necessarily support the greatest amount of growth. Neither does the largest number of individual fruiting structures indicate the most spores. In some cases, including the control, many heads are seen that are greatly reduced in diameter with a resultant reduction in the number of spores. Thus in the case of *C. circinans*, where d-ribose is rated as one of the better sporulation media, only a few heads were present, but they were very large and were estimated to have more spores than where more heads were produced, as on arabinose. In many cases sorbose inhibited both growth and sporulation. Barnett and Lilly (**1951**) had previously reported that sorbose inhibited the growth of *C. cucurbitarum*.

TABLE III

EFFECT OF GLUCOSE/ASPARAGINE RATIOS ON SPORULATION

Glucose/ asparagine ratio	Sporulation ratings*							
	B. trispora		*C. cucurbitarum*		*C. circinans*		*C. conjuncta*	
	NRRL 2456	NRRL 2457	NRRL 2744	NRRL 2745	NRRL 2546	NRRL 2548	NRRL 2560	NRRL 2561
40/2	4	3	0–2	0	2–3	0–3	3	2
20/2	4	3	0–2	0	0–3	0–3	2–3	2
10/2	4	2–3	1–3	0	0–3	0	3	0–3
5/2	4	3	2–3	0	1–2	0	3	2
2/2	3	2–3	1	0–1	1–2	0	4	1–3
0.5/2	3	2–3	4	2–3	2	1–2	4	3
0/2	3	1–3	2	0–2	2	0–3	3	2–3
2/5	3	2	2	0–2	2–3	0–2	4	3
2/3	3	2–3	3	0–2	2–4	0–2	4	3
2/1	4	3	4	0	1–2	0	4	3
2/0.5	4	4	3	0	1–3	0	3	3
2/0	2	2–3	2–3	1	1	0	0–2	0–2

* Ratings: 0 = No sporulation. 1 = 1–10 fruiting heads. 2 = 10–100 fruiting heads. 3 = 100–1000 fruiting heads. 4 = 1000 or more fruiting heads.

This experiment was repeated with glucose, maltose and cellobiose using $(NH_4)_2SO_4$ in place of asparagine. Sporulation of *B. trispora* and *C. conjuncta* appeared to be best on cellobiose although sporulation was also good on the other carbon sources.

A study was also made to determine the best ratio of carbon to nitrogen in all eight strains, using SMA supplemented with various amounts of asparagine and glucose as shown in TABLE III. The plates were incubated at 25° C for one week and the experiment was run on two different occasions. Where variation in the amount of sporulation occurred between runs, this range is given by citing two numbers.

As indicated in TABLE III, sporulation was markedly affected by the amounts of glucose and asparagine used in the medium. All species

formed mycelia which were yellow-colored at the 40-g-glucose level, pale yellow at the 20-g level, and practically without yellow pigment in the remaining asparagine-glucose combinations. With the glucose level as the numerator and the asaparagine level as the denominator, the best combinations for sporulation were: *B. trispora,* 10/2 and 2/0.5; *C. cucurbitarum,* 2/0.5; and *C. conjuncta,* 2/3–5. In general the results with *C. circinans* NRRL 2548 were poor, but after 7 days' incubation, sporangia were most numerous at 2/3. If the plates were held for a longer period, 20–40/2 was best. On media devoid of asparagine, such species as *B. trispora* and *C. cucurbitarum* produce a number of sporangia but no conidia, indicating that sporangia are produced under starvation conditions.

In order to find the optimum temperature for growth and sporulation, mycelial growth from each of the eight strains was seeded on Medium 5 containing either maltose or cellobiose. Plates incubated at 20° C and above were examined after 5 days, whereas those at lower temperatures were read on the 14th day. All plates containing *C. circinans* were read on the 14th day, since this species always sporulates later than others in the family Choanephoraceae. The results indicated no sporulation at 15° C although growth occurred at this temperature. All strains produced only conidia or sporangioles at 20° C except in one instance each with NRRL 2745 and NRRL 2457. A temperature range of 25 to 28° C was best for sporulation. At 32° C sporulation was greatly reduced in every case. No growth occurred at 7° or 42° C, whereas at 37° C all strains except those of *B. trispora* had covered the surface of the agar. At the higher temperatures the mycelia of *B. trispora* was intensely yellow. It would appear that elevated temperatures should be exploited in efforts to increase the yields of beta-carotene. Elevated temperatures tended to suppress the conidial phase of growth and to favor the development of sporangia. With the exception of one strain of *C. conjuncta,* all strains produced only sporangia when grown at 32° C.

It has been reported that light favorably influences the sporulation of *Choanephora cucurbitarum.* We have observed that *B. trispora* grown on SMA plates exposed to light sporulated better and formed more yellow pigment than those placed in the dark. It is not known whether the enhanced pigmentation and sporulation were due to light or to fluctuating temperatures or to a combination of both. To compare the effect of light vs. dark at approximately the same fluctuations in temperature, cultures of the eight strains were inoculated onto PDA in duplicate. One set was exposed to sunlight and the second was placed

in closed, completely dark containers at the same location. Only a few dishes were placed in each container to avoid carbon-dioxide accumulation. *Choanephora cucurbitarium, B. trispora* and *C. conjuncta* all showed better sporulation in light than in total darkness. The effect on *C. circinans* was not so clearly demonstrated even with repeated trials using various media.

Members of the Choanephoraceae, unlike most of the Mucorales, sporulate poorly in pure culture, whereas in nature in mixed culture sporulation is very abundant. To determine whether certain microorganisms might enhance sporulation, pure cultures of yeasts, bacteria and fungi were placed on plates inoculated with each of the four species of the Choanephoraceae. In some cases growth inhibition of the Choanephoraceous strains occurred, especially in the presence of yeasts, wherein the pH of the culture had become quite acid. In other instances, marked increase in sporulation occurred in the area near the test organism. Thus *Alternaria solani* grown on a glucose-salts medium appeared to stimulate sporulation of NRRL 2560. *Streptomyces griseus* NRRL B-150 also stimulated sporulation of both this strain and *C. cucurbitarum*. In most cases, however, no stimulation of spore production was observed with the 11 microorganisms used against strains of each of the 4 test organisms.

SELECTION OF NATURALLY OCCURRING MUTANTS

Of the two mating types of *Blakeslea trispora* which give the highest yields of beta-carotene, the minus strain produces fewer spores than the plus strain. The latter, NRRL 2456, regularly gives uniformly good sporulation and when grown alone contains more beta-carotene than NRRL 2457, as reported by Hesseltine and Anderson (**1957**). It would be advantageous to start the fermentation with equal amounts of spores from the plus and minus strains. We have observed that when lyophil tubes of NRRL 2457 are opened and streaked on SMA, an excellent medium for pigment formation, some of the colonies produce a bright yellow pigment and others appear to have more spores. Colonies that appear high in both can then be selected and relyophilized. It is obvious that by repeating the process of selecting and relyophilizing, colonies might be chosen which would contain higher amounts of beta-carotene and adequate numbers of spores for making inoculum for the fermentation.

Accordingly, a lyophil culture of NRRL 2457 was opened, diluted in broth, streaked onto SMA plates and incubated in direct sunlight at room temperature. From these plates eight selections were made based

on differences in pigmentation and sporulation. When grown on PDA slants, the strains looked very much alike although on SMA there were distinct differences in sporulation and pigmentation. These selections were carried for 10 months, through four transfers on PDA slants, and then again examined on PDA plates. Each grew at about the same rate as the parent and showed approximately the same amount of sporulation, except for one isolate. Pigmentation was similar to the parent except for one nearly white natural variant, 2457-7, which had retained this

TABLE IV

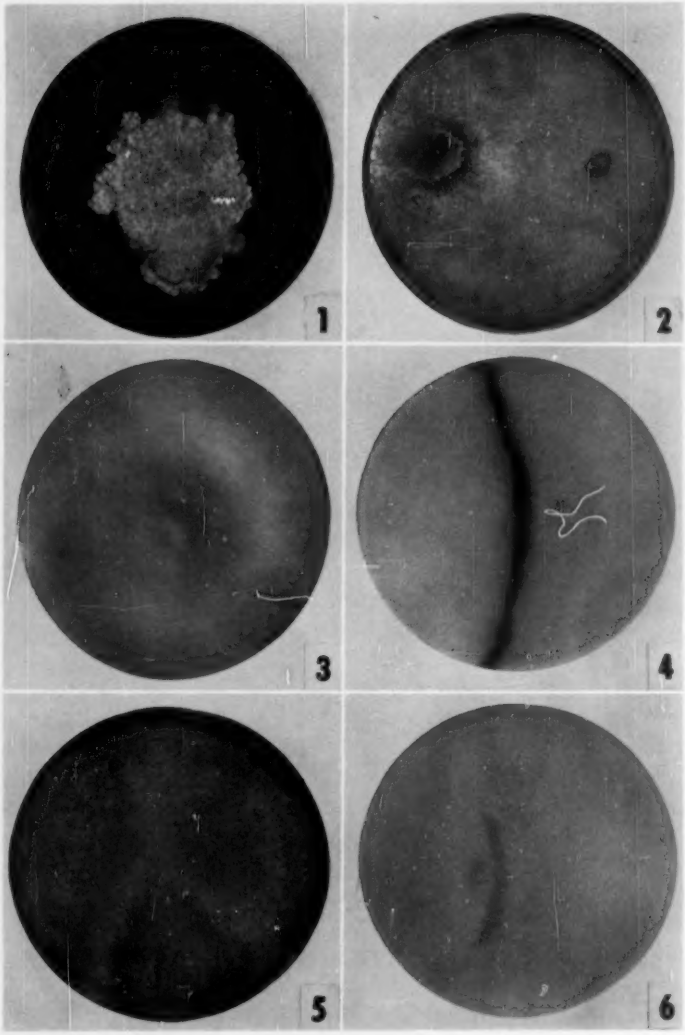
CULTURAL CHARACTERISTICS OF SELECTED ISOLATES FROM C. CIRCINANS, NRRL A-7197

NRRL strain no.	SMA at first transfer			PDA			
	Rate of growth	Pigmentation	Sporulation	Mating reaction	Rate of growth	Sporulation	Aerial mycelium
A-7197	rapid	light yellow	poor	excellent	rapid	none	abundant
A-7197–1	slow	white, in age yellow	none	excellent	rapid	poor	none—colony moist
A-7197–2	slow	white, in age yellow	excellent*	excellent	slow	none	abundant
A-7197–3	growth twice as fast as A-7197–1, –2	orange yellow	excellent	poor	rapid	excellent (4+)	slight
A-7197–4	growth twice as fast as A-7197–1, –2	orange yellow	excellent	poor	rapid	excellent (3+)	slight

* Lost ability to sporulate after several transfers.

colorless characteristic throughout the transfer series. Each of the eight selections was mated on PDA with the opposite mating type, NRRL 2456. One of the variants, 2457-5, formed only a few zygospores at one place where the mated colonies came together, whereas the parent strain gave a heavy dark line of zygospores across the plate from edge to edge. Two variants still produced more yellow color than the parent, and three others showed more sporulation on PDA plates. This technique proved of value in making strain selections of *B. trispora* in that some of them still retained desirable characteristics after several transfers.

A more striking example of how selection gives forms distinct from the parent can be illustrated from a study made on *C. circinans*, A-7197.



FIGS. 1–6.

FIGS. 1–6. *Choanephora circinans*, natural variants. FIGS. 1, 3 and 5 were photographs of mutants grown on SMA for 24 days at 25° C. FIGS. 2, 4 and 6 were the same mutants mated with NRRL 2546 on PDA at 25° C and incubated for 7–10 days. Pictures were taken through the bottom of the Petri dishes.

This culture was sent to us in July, 1957, by Dr. G. W. Martin as *Choanephora* sp. Almost three weeks' incubation on SMA was required before any sporangia at all were produced. The culture was asporous on all other media tested and has remained essentially sterile throughout a series of successive transfers. Although seemingly sterile asexually, this strain formed large numbers of zygospores when mated with NRRL 2546, indicating it was of a strongly minus mating type. From the plate of SMA where sporulation had occurred, lyophil preparations were made and a tube tested for viability by streaking the spores in serum onto SMA. Some of the colonies which developed on these plates grew rapidly and were deep yellow-orange in color; others were slower growing and white. Four isolates were picked and certain characteristics compared with the parent strain as shown in TABLE IV (Figs. 1, 3 and 5).

When strain A-7197-1 was mated with NRRL 2546, zygospore formation occurred as a solid black line completely across the agar in the plate. In contrast, A-7197-4 × 2546 showed only a few zygospores and these in the region where the colonies had first come into contact. The appearance of the mated isolates on PDA is shown in FIGS. 2, 4 and 6. When strain A-7197-4 was relyophilized and streaked on SMA, most of the colonies formed were similar to the original A-7197-4, but a few were sterile like the parent strain, A-7197. Since a reversion to colonies with a sterile, white, aerial mycelium can occur on some media, the plates must be examined carefully to guard against culture rundown. The data listed in TABLE IV under the heading PDA represent the status of the isolates after seven transfers over a period of about $1\frac{1}{2}$ years. The results were similar except that A-7197-2 had lost its ability to sporulate and had reverted back to its parent characteristics. Isolate A-7197-3 after this period tended to sector into several different shades of yellow and orange and also exhibited variable amounts of sporulation.

DISCUSSION

The family Choanephoraceae contains two genera, *Choanephora* and *Blakeslea*, which are closely related to each other in many respects.

FIG. 1. Mutant A-7197-2. The colony has an irregular margin and the darkened regions are areas of heavy sporulation. FIG. 2. NRRL 2546 × A-7197-2. Notice the broad band of zygospores about the restricted growth of the mutant. FIG. 3. Mutant A-7197-1. The colony lacks sporulation but shows a large amount of white, sterile aerial mycelium. FIG. 4. NRRL 2546 × A-7197-1. Notice the heavy line of zygospores similar to that seen when the parent strain, A-7197, was mated with NRRL 2546. FIG. 5. Mutant A-7197-4. The colony is low, spreading and has excellent sporulation. FIG. 6. NRRL 2546 × A-7197-4. Notice the short diffuse line of zygospores which has not extended across the plate.

Their ecology in nature and their habit of growth in pure culture are similar, as are the morphology and development of their sexual stages and their manner of asexual sporulation (Hesseltine, **1953**; Hesseltine and Benjamin, **1957**). Physiologically, they have in common the characteristic of producing carotenes in increased yields when (+) and (−) strains are combined in liquid media. This production has been demonstrated in *Choanephora cucurbitarum* by Barnett et al. (**1956**) and in *C. circinans, C. conjuncta* and *Blakeslea trispora* by Hesseltine and Anderson (**1957, 1958**). The latter workers also showed that interspecific, as well as intraspecific, combinations of mating types belonging to the same genus or to different genera bring about the enhanced production of beta-carotene.

Although *Choanephora cucurbitarum* has been studied from the standpoints of the effect of light and the utilization of carbon and nitrogen sources, there has been no attempt to study the various species of *Choanephora* and *Blakeslea* in a comparative manner. The results we have obtained further confirm the close relationship of the members of the family Choanephoraceae. Thus, each of the four species can use ammonium nitrogen, urea and asparagine, but none can utilize nitrate or nitrite (TABLE I). With respect to the carbon sources tested (TABLE II), all four species utilize l-arabinose, glucose, galactose, trehalose, cellobiose and maltose, but not i-erythritol, d-arabinose, l-sorbose, melibiose, lactose, sucrose or raffinose. These results both confirm and extend the findings of previous workers. The variable utilization of soluble starch by our strains probably indicates that starch cannot be used to any considerable extent as such, but that a small amount was hydrolyzed, accounting for the slightly positive or positive responses. In most cases d-xylose is not readily attacked and a period of adaptation is required before this pentose is utilized.

In general we found that a low concentration of carbohydrate and temperatures of about 25° C favored asexual spore production. An exception is found in *C. circinans,* where sporulation was better, although delayed, by the use of higher carbohydrate concentrations. It should be noted that at the higher glucose levels more intense yellow-orange colors were produced. An increase in temperature up to 37° C enhanced pigmentation, which suggests that elevated temperatures and increased amounts of carbohydrates might well be used to advantage for the production of carotenes. In our experience there is a great deal of strain variation with respect to sporulation, so that a sporulation medium recommended for one strain of a species may not be suitable for all isolates of that particular species. For example, the medium suggested by

Barnett and Lilly (**1950**) is excellent for their strains of *Choanephora cucurbitarum*, but was unsatisfactory for some wild strains which we isolated.

In addition to substrate and temperature factors, the selection of natural mutants can be used to alter sporulation and carotene content in cultures of *Choanephora* and *Blakeslea*. Using the technique described earlier, one can easily select isolates with greatly increased sporulation, pigmentation, or both. Since some of these isolates appear to remain stable for a considerable length of time, one could select strains suitable for use in fermentations to produce higher initial beta-carotene yields or better sporulation for inocula. Presumably with properly induced mutations the number of desirable mutants could be greatly increased. One might also expect that mated (+) and (−) strains which individually produce large amounts of beta-carotene would give higher yields in a fermentation than would mated strains which individually produce lesser amounts.

SUMMARY

Unmated (+) and (−) strains of all four available species of *Choanephora* and *Blakeslea* were studied in respect to such growth and sporulation factors as nitrogen sources, carbon sources, carbon-nitrogen ratios, temperature, light, and the influence of other organisms. With glucose as the carbon source, ammonium sulfate and asparagine were the best of five sources of nitrogen tested. When asparagine served as the nitrogen source, glucose and cellobiose were the best of 17 carbon compounds tested. Carbon-nitrogen-ratio requirements differed among species. Cultures generally sporulated best at temperatures of 25–28° C and under alternating periods of light and darkness. Sporulation was not generally enhanced by the presence of other microorganisms. Some of the natural variants selected from plate cultures of *B. trispora* and *C. circinans* still retained their individuality after 7 transfers over a period of 18 months.

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INDUCED DIMORPHISM IN DERMATOPHYTES [1, 2]

JOHN W. RIPPON [3] AND GEORGE H. SCHERR

(WITH 8 FIGURES)

Most pathogenic fungi can be conveniently divided into two groups: those causing superficial infections, such as *Trichophyton* and *Microsporum* species, and those invading the deeper organs, such as *Histoplasma* and *Blastomyces*. In reviewing the dimorphism phenomenon in yeasts, Scherr and Weaver (**1953**) concluded that the ability of a fungus to invade deep tissues usually depends upon the capacity of the organism to exist in or have the potential for yeast-phase growth. This is, in part, apparently a consequence of the environmental conditions imposed by the host (Ainsworth, **1955**; Scherr and Weaver, **1953**). The superficial fungi, on the other hand, are characteristically mycelial in their infective stage (Ainsworth, **1955**; Saez, **1957**). Between these two groups are pathogens with limited capacity for the invasion of deeper tissues, such as the etiologic agents of chromoblastomycoses and sporotrichosis.

If a dermatophyte could be induced to assume a morphological and physiological state more consistent with that of the dimorphic pathogenic fungi, the organism might concomitantly show an altered pathogenicity for deep organs.

In recent literature several instances of "deeper" invasion by superficial dermatophytes have been noted. Wilson et al. (**1954**) and Cremer (**1953**) reported the dermatophyte *Trichophyton rubrum* as causing nodular granulomatous lesions similar to Majocchi's granuloma. Symeonidis and Emmons (**1955**) described "numerous hyphae in the form of irregular tubes with swellings and constrictions" in their report of

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[2] A portion of this report was presented at the X International Congress of Genetics, Montreal, Canada (Rippon and Scherr, **1958**).

[3] This report is taken from a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Illinois Professional Colleges.

a mucormycosis. In case reports of rarer mycoses Emmons et al. (**1957**) described "swollen spherical fungus cells" in a human infection of *Basidiobolus;* Binford et al. (**1952**) reported a brain abscess of *Cladosporium trichoides* in which "bulbous swelling" of the mycelium was found; and "distorted" hyphal fragments, bulbous in nature, were noted in a case of *Cercospora apii* by Lie-Kian et al. (**1957**).

Infection has also resulted from fungi considered saprophytic, and in the described cases of aspergillosis, penicilliosis and mucormycosis as noted in Conant's Manual (**1954**), distorted mycelial elements were occasionally observed.

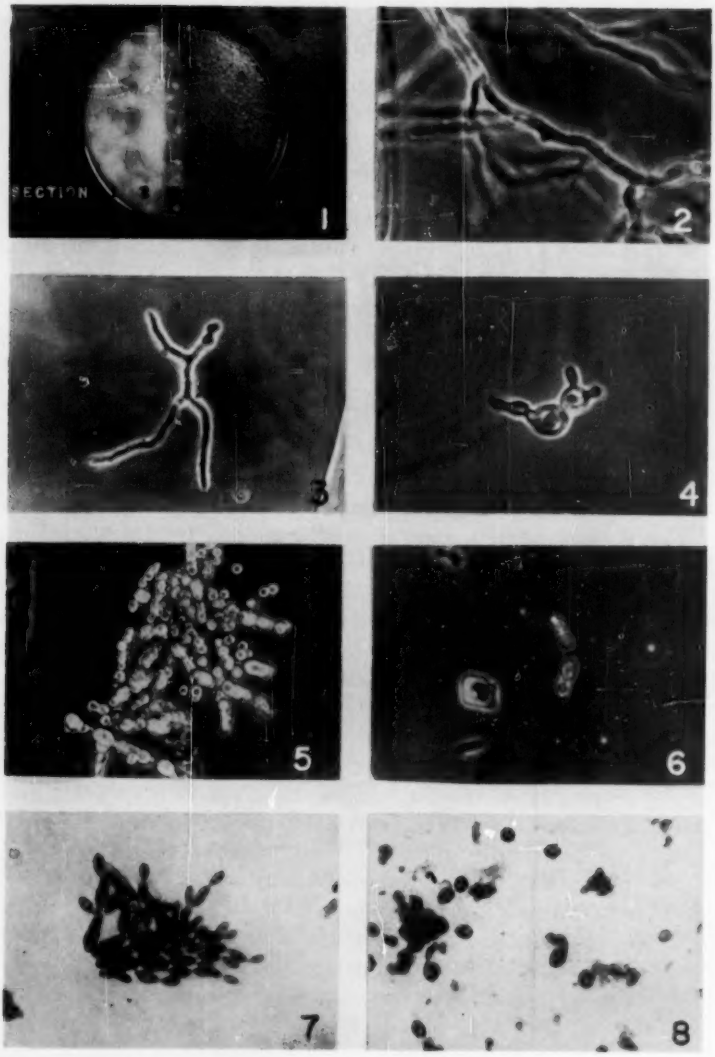
Several investigators have studied the factors influencing yeast-mycelium conversion of the normally dimorphic fungi (Scherr and Weaver, **1953**; Ainsworth, **1955**; Scherr, **1957**; Nickerson, **1957**; and Pine and Peacock, **1958**). Some of the conditions which were generally found important included incubation temperature, simple chemicals having −SH groups, and miscellaneous growth factors supplied by the host body. The dependence of pathogenicity on this conversion phenomenon was demonstrated by Winsten and Murray (**1956**) when a mycelial, non-pathogenic mutant of *Candida albicans* (806 of Mackinnon, **1940**) was converted to a yeast phase by being grown in a medium containing cysteine, and thus regained its invasive ability for mice. Recently, investigations into the inducement of the parasitic phase with the organisms of chromoblastomycosis were carried out by Silva (**1957**) with the production of "sclerotic" cells from *Phialophora* species.

Experimental work on the pathogenicity of dermatophytes for deep tissue was attempted by Sulzberger (**1929**) and Brocq-Rousseau et al. (**1926**) with negative results. Sternberg et al. (**1952**) injected suspensions of *Trichophyton rubrum* into mice and observed granulomatous masses on the liver, spleen and omentum in a few mice on autopsy, and an occasional unicellular "spherule."

The work presented in this paper concerns the *in vitro* and *in vivo* inducement of morphological changes, simulating dimorphism, in the dermatophytes *Trichophyton rubrum, Microsporum audouini,* and *Cladosporium mansoni,* associated with a concomitant increase in invasive ability for the deep tissues of experimental animals.

MATERIALS AND METHODS

Cladosporium mansoni (ATTC 7371), *Trichophyton rubrum* and *Microsporum audouini* were used in this study, the latter two being procured from clinical cases of tinea capitis at the University of Illinois



FIGS. 1–6, 8. *Trichophyton rubrum;* FIG. 7. *Microsporum audouini.* FIGS. 2–8, × 440; 2–6, phase microscope. FIG. 1. Fungus on cysteine-gradient plate, indicating the sections scraped and used as inoculum for pathogenicity tests. Section 1 has the lowest cysteine concentration. FIG. 2. Wet mount of inoculum from section 1 of cysteine plate, showing essentially normal mycelium. FIG. 3. Wet mount of

Dermatology Clinic and Fantus Clinic of Cook County Hospital, Chicago. Stock cultures were maintained on Sabouraud's glucose agar.

Preparation of media for in vitro studies. The basal medium, a modified Swartz and Georg (**1955**) medium, consisted of 50 ml of 10% hydrolysed casein, 40 g glucose, 15 g agar, 0.1 g $MgSO_4$, 0.5 ml trace elements, 200 ml Sorensen buffer at pH 6.8 and distilled water q.s. to 1000 ml. Gradient tilt plates were prepared using 150 mm Petri dishes in which the upper layer consisted of the basal medium to which cysteine had been added to give a final concentration of 0.1 M.

Growth on cysteine-gradient plates. Two twelve-day-old cultures of each of the dermatophytes, grown on Sabouraud's slants, were scraped, and suspensions prepared using a total of 10 ml saline. The inocula were checked microscopically for typical morphology. Two ml of these saline suspensions were distributed evenly over the surface of the cysteine-gradient plates, and incubated at 37° C for 3 weeks. Growth of the organisms occurred over approximately one half of the gradient plates. Comparisons were run using gradient plates made of Sabouraud's glucose agar as the basal medium; no differences were observed. The area of growth on the plates was divided into three sections perpendicular to the gradient axis (FIG. 1). Growth was scraped from each section and shaken with glass beads for 30 minutes. The suspensions were centrifuged, washed, and one half of the centrifugate was suspended in 5% hog gastric mucin, the other half in saline. Control suspensions from normal growth on Sabouraud's slants were also made. All the suspensions were diluted to approximately equal turbidity.

Animal studies with mice. Male Swiss albino mice (30–35 g) were divided into six groups of six mice each. Two groups were injected with inocula from each plate section, receiving respectively organisms suspended in saline and in gastric mucin. Each mouse received 0.5 ml of the suspensions intraperitoneally. Groups of mice were usually autopsied at 10 and 16 days post inoculation.

Animal studies with rabbits. Twelve-day-old stock cultures of the dermatophytes grown on Sabouraud's glucose agar were harvested as

inoculum from section 2 of cysteine plate. Bulbous mycelial forms more apparent. FIG. 4. Wet mount of inoculum from section 3 of cysteine plate, showing thick-walled budding form. FIG. 5. Branched series of round bodies after 16 days incubation in a cellophane bag implanted in the rabbit peritoneum. Wet mount. FIG. 6. Unicellular budding forms after 34 days incubation (in bag) in rabbit peritoneum. Wet mount. FIG. 7. *M. audouini,* rounded bulbous forms after 20 days incubation (in bag) in rabbit peritoneum. Hotchkiss-MacManus stain. FIG. 8. *T. rubrum* in Hotchkiss-MacManus stain of excised shoulder lesion, showing unicellular budding forms.

noted above. Five ml of these saline suspensions were placed in dialysis tubing and tied at both ends to form a sealed bag. These bags were sterilized previous to inoculation by being soaked in 70% alcohol and washed in sterile distilled water. The bags were implanted into the peritoneal cavity of rabbits, under nembutal, and secured to the skin of the animal by silk thread to prevent migration. Bags were removed at periods from 12 to 34 days post implantation and aliquots of their contents were examined under wet mount, cultured, Hotchkiss-MacManus stained, and tested for pathogenicity in mice and rabbits.

Suspensions of the organisms recovered from the bags were injected into the shoulders of the rabbits in which the bags had been implanted and into the shoulders of the control rabbits. Aliquot portions of the bag washings were also injected intraperitoneally into adult male Swiss albino mice. Control mice were injected with normal organisms grown as Sabouraud's glucose agar slants, in suspensions approximately equal in turbidity to those of the altered organisms. Each mouse received 0.5 ml I.P. of suspension. Groups of mice were autopsied at 14 and 17 days post injection.

RESULTS

At the lowest concentrations of cysteine on the gradient plates, the mycelial elements of *T. rubrum* and *M. audouini* appeared mostly normal (FIG. 2). As the concentration in the gradient increased, the hyphae became more and more distorted (FIG. 3). Growth ceased a little under half way across the gradient. In the area just preceding cessation of growth, bulbous rounded forms and monocellular "spherules" predominated. The rounded forms were thick-walled, and often displayed multiple budding (FIG. 4). Essentially the same appearance was noted for both *M. audouini* and *T. rubrum*. Grossly, the fluffy colonial morphology became more compact as the cysteine concentration increased, and at the outer edge of growth the colonies were extremely tenacious and moist.

Associated with the morphological changes were changes in pigmentation. *M. audouini* displayed variations from the normal salmon color to reddish salmon, intense red, and finally no pigment, in bands perpendicular to the gradient axis. The pigment of *T. rubrum* varied from deep red to greyish yellow, with no pigment in the last third of the area of growth. *C. mansoni*, which is normally a black-pigmented, filament-forming yeast-like organism, grossly compact, lost its black pigment and became entirely yeast-like in appearance at the outer

edge of growth. Bands of lighter gray pigmentation appeared across the plate.

The data in TABLE 1 indicate that *T. rubrum* harvested from these gradient plates showed an increased pathogenicity as manifest by lesions in the infected animals coincident with their isolation from areas on the

TABLE 1

INCREASE IN PATHOGENICITY OCCURRING WITH ALTERATION OF MORPHOLOGY

Mice injected with suspensions of *T. rubrum* from sections of cysteine-gradient plate (see FIG. 1). Summary of gross autopsy results

Plate section	Days post injection	Autopsy results	
		Organisms suspended in saline	Organisms suspended in mucin
1	10	a. lesion at site of injection b. lesion at site of injection and on liver c. negative	a. negative b. negative c. negative
	16	a. negative b. negative c. negative	a. negative b. negative c. negative
2	10	a. negative b. negative c. adhesion on liver *pos. on smear	a. negative b. negative c. died on day of injection
	16	a. pos. lesions on liver b. pos. lesions on liver and spleen c. negative	a. negative b. negative c. died on day of injection
3	10	a. pos. lesions on liver b. pos. lesions on liver c. pos. lesions on liver	a. pos. lesions on liver b. pos. lesions on liver c. pos. lesions on liver
	16	a. negative b. pos. lesions on liver c. pos. lesions on liver and spleen	a. pos. lesions on liver and kidneys b. pos. lesions on liver and spleen c. pos. lesions on liver and spleen

* Positive results were determined by Hotchkiss-MacManus stain of lesion material and reculture of the organism.

plate containing the higher concentrations of cysteine (plate section 3 in FIG. 1). Occasional lesions were found in mice inoculated with organisms harvested from the area on the plate having the lower concentration of cysteine (section 1). These tended to be mycelial masses lying on the surface of organs. In mice inoculated with organisms from section 2, lesions were more prevalent than those inoculated with

organisms from section 1. Invasion of the organs themselves was widespread in the third group of mice. The liver, kidneys, and omentum contained lesions of the mycotic-granuloma type. Hotchkiss-MacManus stains of squash smears made from the internal organs showed masses of single-celled, multiple-budding, spherule-like forms and bulbous, constricted short hyphal elements in compact masses within the tissue. No difference was apparent between animals injected with material suspended in saline or gastric mucin. It is apparent that the increase in pathogenicity of organisms harvested from the three different sections on the gradient plates was accompanied by a morphological transition to yeast-like forms.

These findings were confirmed in repeated experiments. *T. rubrum* and *M. audouini* harvested from the third section of the cysteine plates and injected into mice showed extensive involvement of the diaphragm as well as other organs. Occasionally the spleen was obliterated and a massive granuloma was noted involving most of the peritoneal region.

In-vivo altered organisms. The most striking morphological changes were manifested by *Trichophyton rubrum* in the bags removed from rabbits. On the twelfth day, growth of the inocula had occurred with a tendency to rounded bulbous forms. By the sixteenth day the predominant findings were of numerous round bodies in branched series (FIG. 5) and some unicellular budding forms. In bags removed after periods of time greater than sixteen days post implantation, the single-celled forms were more and more numerous. On the thirty-fourth day the unicellular forms were seen almost exclusively (FIG. 6). In other words, with increased incubation in the intraperitoneal cavity of the rabbit there was more pronounced metamorphosis to yeast-phase growth. On subculture, the altered forms immediately gave rise to the normal mycelium when grown at 25° on Sabouraud's slants.

The effect of the rabbit *in vivo* environment on *Microsporum audouini* required a longer time to achieve results comparable to those of *Trichophyton rubrum*. Distorted mycelium appeared on the twelfth and sixteenth days post implantation, similar to that observed on the gradient plates. By the twentieth day the organism had assumed a rounded bulbous form in series (FIG. 7). No unicellular forms were noted. Normal mycelium appeared readily on reculture at 25° on Sabouraud's slants.

Cladosporium mansoni removed from the intraperitoneal bags appeared as a yeast form similar to that noted on the cysteine-gradient plates. With reculture at 25° on Sabouraud's medium, the normal colonial appearance was observed.

In all cases, controls were run with suspensions of normal organisms. In no case was infection with the normal control observed. *Trichophyton rubrum,* altered by incubation in the rabbit, when injected into the shoulders of rabbits, caused the formation of subcutaneous lumps in three of the four animals. The shoulders of these rabbits were excised, and the tissue from these lesions on Hotchkiss-MacManus slides showed numerous yeast-like organisms (FIG. 8), which were recultured as normal mycelium. The subcutaneous lumps were resolved by the eighteenth day post injection. However, an erythema appeared in the shoulder site of injection, of the dermatitis type, from which mycelial *T. rubrum* was recultured. This did not occur in the controls injected with suspensions of normal organisms.

The results with mice injected with *in-vivo* altered *T. rubrum* were more vivid. On the fourteenth and seventeenth days post injection, numerous lesions were found on the liver, several on the kidneys, and also throughout the omentum. In one case an open lesion existed at the site of injection. Hotchkiss-MacManus slides showed the lesions to consist primarily of unicellular forms. *T. rubrum* was recultured from the lesions. In no case were lesions observed with normal controls.

Smaller subcutaneous shoulder lumps in rabbits were found when altered *Microsporum audouini* was injected. These resolved by the tenth day post injection. Areas of erythema similar to those noted with *T. rubrum* were observed, and the organism was recultured. Again this did not occur with the normal control.

A few lesions were found in mice injected with the altered *M. audouini.* The appearance of the organism in tissue was similar to the organisms recovered from the I.P. bags.

Cladosporium mansoni caused large purulent lesions when injected into the shoulders of rabbits. These lesions occasionally became open sores with secondary bacterial invasions.

C. mansoni was recultured from excised material. By the twenty-fifth day post injection the lesions were resolved. No lesions were noted in controls injected with unaltered organisms.

DISCUSSION

A type of morphological alteration in dermatophytes resembling dimorphism was induced *in vivo* and *in vitro,* and such alteration resulted in an increase in pathogenicity as manifest by invasion of deep tissue. The appearance of the experimentally altered organisms resembled that generally attributed to the yeast phase of some of the dimorphic fungi and related organisms such as the etiologic agents of chromoblasto-

mycosis. Our results indicate that, under certain conditions, the dermatophytes *Trichophyton rubrum, Microsporum audouini* and *Cladosporium mansoni* can survive, proliferate and cause disease within the animal body. This is in apparent contradiction to the results recently reported by Lorincz et al. (**1958**) working with *Trichophyton mentagrophytes*. They found that suspensions of this dermatophyte implanted intraperitoneally in mice did not proliferate but could be recultured. Similarly, when the organisms were cultured in media containing fresh serum, growth was inhibited. These results were interpreted as evidence for a humoral mechanism inhibitive to dermatophytes. Ayers and Anderson (**1934**) earlier reported a humoral component from patients with "Phytid" eruptions which inhibited dermatophyte growth in culture. However, Lewis and Hopper (**1948**) could not duplicate their results. In the present work, no direct evidence of an *in vivo* nonspecific inhibitory factor could be presented but no proliferation of the organisms occurred if the normal mycelium was injected into experimental animals. When the mycelium was altered previous to injection by growth on cysteine plates, not only did the organisms readily proliferate *in vivo* but they also invaded the tissues. We conclude from this that the mycelial phase of these fungi (dermatophytes) cannot readily multiply within the environment imposed upon it by the conditions of the host. This is consistent with prior observations made *in vitro* by Scherr (**1957**) that the mycelial phase of *H. capsulatum* would be inhibited by a certain concentration of −SH groups or a reduced O−R potential, or both at 37°, these same conditions showing no deleterious effect on the yeast phase of this pathogen.

If the dermatophyte is protected from certain of the antagonistic elements *in vivo* by being semi-isolated in a plastic bag, then in time a gradual metamorphosis takes place to a cultural phase (yeast-like) whose environmental optima are more consistent with those of the animal host. When this happens these organisms can readily multiply *in vivo* and invade tissue. These observations are consistent with our findings here and also with those of Winsten and Murray (**1956**) who worked with *C. albicans*. The fact that mycelial elements of *C. albicans* can be found in infected tissues is not inconsistent with our thesis that the potentiality of yeast-phase growth or the yeast phase itself is essential for invasion of tissues by these organisms. It has been demonstrated, at least for *H. capsulatum* (Scherr, **1957**), that the O−R potential of a grown yeast-phase culture is compatible with growth of the M phase but that the O−R potential necessary to initiate the growth of such a Y phase is not compatible, and in fact very inhibitory, to the M phase. Thus Scherr

concluded "that once the O−R potential of the medium (among other conditions) has determined the phase of the organism, the metabolic rate of the organism would then determine the O−R potential of the medium."

In pathogenicity tests, no difference was observed between normal and pre-exposed rabbits (those having had intraperitoneal bags of the organism) when injected with the altered organisms, suggesting no specific immunological effects under the experimental conditions used.

The factors found important for *in vitro* alteration of dermatophytes are essentially those noted for normally dimorphic fungi. In other experiments the necessity of 37° incubation temperature for the most vivid morphological alteration to yeast-like growth of the dermatophytes on the cysteine plates was demonstrated. The effect of labile sulfhydryl groups seems quite specific and is not entirely a consequence of the lowered Eh of the media (Rippon, **1957**). Distorted, bulbous mycelium was found when *M. audouini* was grown in a medium with Eh buffers of cysteine on thioglycollate but no alteration of morphology occurred when the same Eh was attained with other buffers. The importance of labile −SH groups for the dimorphism in *C. albicans* was demonstrated by Nickerson (**1957**) and was postulated to be a general phenomenon of morphogenesis in fungi.

Several investigators have recultured dermatophytes from blood and tissue of animals thirteen days post injection of spore and mycelial suspensions of the organisms (Saeves, **1916**; Sulzberger, **1928**, **1929**; Catanei, **1945**), indicating that these organisms can exist within the animal body and on its tissue. As a result of or associated with the morphological alteration in the dermatophytes tested in the present study, there is an increased ability for invasion of deep tissues. It is necessary to emphasize that the experimentally altered organisms were found not only on the surface of the organs but in the tissue. The appearance of the mouse liver following injection of the *in vivo* or *in vitro* altered dermatophytes somewhat resembled the mycotic granuloma of candidiasis (Salvin et al., **1952**) and coccidiomycosis (Tarbet, noted in Sternberg et al., **1952**). The developing lesions grew and invaded adjacent tissue, numerous nodules being found throughout the body cavity. There was no tendency for the lesions in the mice to resolve during the course of the experiment, as the severity of involvement increased with time.

The ease with which *T. rubrum* can be induced to assume a tissue phase confirms the contention of Sternberg et al. (**1952**) and Lewis et al. (**1952**) that this organism should perhaps be placed with *C. albicans*

in an intermediate classification of groups where invasion of deep tissue is possible under certain conditions.

The present work indicates that the postulated conversion of a superficial organism into a deep-invading organism (Scherr and Weaver, **1953**) by inducing a morphological change simulating dimorphism can be accomplished in the laboratory. Thus the mode by which the "occasional" mycotic pathogens adapt to in-tissue existence can be more fully elaborated. In this artificial system of producing deep-invading fungi from dermatophytes, specific agents aimed at preventing this conversion could possibly find application in the treatment of disease caused by the dimorphic fungi. Such studies are presently under way.

SUMMARY

The dermatophytes *Trichophyton rubrum, Microsporum audouini* and *Cladosporium mansoni* were subjected to increasing concentrations of cysteine on gradient plates. A type of morphological alteration resembling mycelial-to-yeast dimorphism occurred with increasing levels of cysteine. Associated with this morphological alteration was an increase in pathogenicity as manifested by invasion of the deep organs in experimental animals.

A similar type of morphological alteration and associated increase in pathogenicity was obtained when these organisms, contained in dialysis bags, were incubated in the intraperitoneal cavity of rabbits.

These experiments demonstrated a direct correlation between conversion of the dermatophytes to yeast-phase growth and pathogenicity for deep tissue.

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NOTICE

Because of increased costs in labor, paper, presswork, etc., over the past five years, it is necessary to increase the price of reprints. Beginning with the January–February, 1960, issue of MYCOLOGIA the price of reprints will be as indicated on the inside back cover of this issue.